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Exposure to multiple mycotoxins, environmental enteric dysfunction and child growth: Results from the AflaCohort Study in Banke, Nepal

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Abstract

Evidence of the impact of exposure to multiple mycotoxins and environment enteric dysfunction (EED) on child growth is limited. Using data from a birth cohort study, the objectives of this study were to (a) quantify exposure to multiple mycotoxins (serum aflatoxin [AFB_1] and ochratoxin A [OTA], urinary fumonisin [UFB_1] and deoxynivalenol [DON]), as well EED (lactulose:mannitol [L:M] ratio); (b) examine the potential combined effects of multiple mycotoxin exposure and EED on growth. Multivariate regressions were used to identify associations between growth measurements (length, weight, anthropometric *z*-scores, stunting and underweight) at 24–26 months of age and exposure to mycotoxins and EED at 18–22 months ($n = 699$). Prevalence of AFB_1, DON, OTA and UFB_1 exposure ranged from 85% to 100%; average L:M ratio was 0.29 ± 0.53. In individual mycotoxin models, AFB_1 exposure was negatively associated with weight, WAZ, increased odds of stunting (odds ratio [OR]: 1.28, 95% confidence interval [CI]: 1.08, 1.52; $p = 0.004$) and underweight (OR: 1.18, 95% CI: 1.00, 1.38; $p = 0.046$). Irrespective of other mycotoxin exposure and presence of EED, AFB_1 was negatively associated with length, weight, head circumference, LAZ and WAZ, and with increased odds of stunting and underweight, UFB_1 was associated with increased odds of underweight, and DON was negatively associated with head circumference. EED was associated with the impaired length and weight. These findings suggest that certain mycotoxins and EED may have independent impacts on different facets of growth and that aflatoxin dominates such impacts. Thus, programs reducing exposure to mycotoxin and EED through multi-sectoral nutrition-sensitive interventions have the potential to improve child growth.

KEYWORDS
aflatoxin, deoxynivalenol, fumonisin, growth, ochratoxin, stunting, underweight

1 | INTRODUCTION

Growth faltering can have long-lasting detrimental impacts on both long-term physical growth and cognitive development, particularly in low- and middle-income countries (LMICs). Nepal has registered one of the most dramatic reductions in stunting (i.e., low height-for-age) and wasting (i.e., low weight-for-height) rates in the past three decades (Poudel Adhikari et al., 2021). However, progress has recently stalled, and both stunting and wasting remain serious public health problems in Nepal, with average national stunting and wasting rates at 36% and 10%, respectively (Ministry of Health [MOH/Nepal], 2017). While many risk factors contribute to poor nutrition, previous studies suggest that mycotoxin exposure may be an important factor working independently and together with other risk factors (e.g., low maternal stature, female smoking, low-dietary diversity, inadequate hygiene and sanitation, etc.; Prentice et al., 2013; Wild et al., 2015).

Mycotoxins are natural food-borne toxins, consisting of low-molecular-weight metabolites produced by fungi (Wild et al., 2015). The International Agency for Research on Cancer (IARC) classifies toxic agents into five groups (Group 1, 2A, 2B, 3 and 4) according to existing scientific evidence for their carcinogenicity (IARC, 1993). The IARC classifies aflatoxin B1 (AFB_1) as carcinogenic to humans (Group 1), ochratoxin A (OTA) and fumonisin B1 (FB_1) as possible human carcinogens (Group 2B), and deoxynivalenol (DON), as Group 3 (not classifiable in terms of its carcinogenicity to humans), because data are too limited, inadequate or inexistent (Ostry et al., 2017).

Contamination by mycotoxins can occur at the farm level before harvest, during drying postharvest, in storage and/or during food processing. Many of the poorest people in LMICs are exposed to these natural toxins on a daily basis, particularly through eating diets heavily reliant on groundnuts, maize, and other cereals (Wild et al., 2015). Infants are often given complementary foods at a young age, typically made from household staples such as cereals that are vulnerable to fungi that produce mycotoxins.

Although precise causal pathways explaining the association between aflatoxin, fumonisin and DON and poor linear growth are unknown, one hypothesis is of mycotoxin-induced enteropathy. It has been postulated that the mechanism by which mycotoxins affect growth could be through increasing environmental enteric dysfunction (EED), which, in turn, affects growth (Smith et al., 2012). EED is an incompletely defined syndrome of gut inflammation, reduced absorptive capacity and reduced barrier function in the small intestine (Crane et al., 2015; Smith et al., 2012, 2017). Moreover, while several studies have linked AFB_1 to poor linear growth among children in LMICs (Gong et al., 2003, 2004; Hoffmann et al., 2018; Turner et al., 2007; Watson et al., 2018) only a few have been conducted to examine links between groups of mycotoxins and child growth (Chen et al., 2018; Lombard, 2014; Shirima et al., 2015). Current mycotoxin regulations are mainly based on toxicological data of an individual mycotoxin exposure at a time and do not account for the potential combined effects of exposure to multiple mycotoxins simultaneously, or indeed to potential interacting risk factors such as EED (M. C. Smith et al., 2016). Given the concurrent contamination of multiple mycotoxins across commonly consumed foods and commodities, there is a need to ascertain the extent of these combined effects on child growth (Tesfamariam et al., 2020).

Key messages

- Mycotoxin exposure was highly prevalent in children aged 18–22 months. Various mycotoxins and EED contribute independently to different manifestations of poor child growth
- AFB1 was negatively associated with length, weight, head circumference, LAZ, as well as WAZ, and associated with increased odds of stunting and underweight. UFB1 was also associated with increased odds of underweight. DON was negatively associated with head circumference.

The objectives of this study were to (a) quantify coexposure to multiple mycotoxins and EED (as measured by lactulose:mannitol [L:M] ratio); (b) examine the combined effects of EED and mycotoxin exposure on child growth.

2 | MATERIALS AND METHODS

2.1 | Study design, setting and study subjects

Data for this study are from the AflaCohort Study, a prospective birth cohort study conducted in Banke, Nepal, from 2015 to 2019 (Andrews-Trevino et al., 2020). The AflaCohort Study followed 1675 mother–child dyads longitudinally during the first 1000 days to examine temporal relationships between aflatoxin exposure and child growth impairment, particularly child length-for-age *z*-score. Women were first visited during gestation and again at birth. Women and their children were visited at predetermined intervals—that is, 3, 6, 9, 12, 18–22 and 24–26 months after delivery (Figure S1).

Women were eligible to participate if they were: 16–49 years old; less than 30 weeks of gestation (by the woman's estimate); had no plans to move out of the study area throughout the study period; planned to deliver in the study area; provided written informed consent herself or through a legal guardian; and had a singleton, live birth. Women were excluded if they were severely malnourished (mid-upper arm circumference [MUAC] < 17.5 cm), had severe anaemia (Hb < 7.0 g/dl) or had pregnancy-induced hypertension. Children were excluded if they were severely malnourished (defined as weight-for-length (WLZ) *z*-score ≤ −3 at 3 months of age, MUAC < 11.5 cm at 6–26 months, or bilateral pitting oedema), had severe anaemia (Hb < 7.0 g/dl), were born with congenital anomalies, were born very-low-birthweight (<1500 g) or suffered from sepsis or respiratory distress syndrome.

In the first phase of the study, from pregnancy until the child turned 1 year of age (2015–2018), the research team collected one maternal and three infant blood samples for AFB_1 biomarker (AFB_1-lysine) testing. During the second phase when with children

18–26 months of age (2018–2019), the team collected serum and urine samples to measure mycotoxin exposure and EED at 18–22 months of age. More specifically, samples were collected from 699 of the children to measure exposure to multiple mycotoxins (including aflatoxin) as well as concentrations of lactulose and mannitol as a measure of EED. Children who did not meet the 18–22 months of age eligibility criteria (i.e., older than 22 months of age when the second add-on phase of the study launched) were excluded from the biological sample collection. Collecting biological specimen data from children ages 18–22 months and anthropometric data at the follow-up visit (children ages 24–26 months of age) ensured temporal sequencing between the exposures of interest (mycotoxins and EED) and the outcomes of interest (anthropometric measurements).

This analysis draws primarily on data from the second phase of the study, with the exception of using select maternal characteristics (i.e., maternal education level) from the visit during pregnancy, and birth (or 3 months) anthropometric data as control variables in the regression models described below.

2.2 | Ethical oversight

The Nepal Health Research Council and the Tufts Institutional Review Board approved the study, as did the Family Welfare Division of the Ministry of Health and Population and the Banke district public health office. The women (or their legal guardians) gave verbal and written consent before participation.

2.3 | Blood sample collection at 18–22 months of age

Haemoglobin (Hb) tests were administered before the venipuncture using a portable HemoCue Hb 301 (HemoCue Inc.). Children found to have anaemia (Hb < 11 g/dl) were referred to the appropriate health facility or personnel for treatment. Blood draws were not performed if children were found to be severely anaemic (Hb < 7.0 g/dl).

After ensuring eligibility to proceed for a venous blood draw, nurses performed paediatric dorsal venipuncture to collect 1–3 ml of blood from the children. Five millilitres BD Vacutainer® blood collection tubes and 23 gauge butterfly needles were used to collect blood samples from the children. Blood samples were tested for AFB_1 and OTA.

2.4 | Urine sample collection at 18–22 months of age

EED was measured using a urinary L:M dual sugar absorption test. This is a noninvasive proxy marker commonly used as an alternative to histopathological methods, such as small intestinal biopsies, used to diagnose EED (Denno et al., 2014). The local laboratory technician prepared batches of 1000 ml of L:M solution consisting of 625 ml of sterile distilled water, 375 ml of lactulose (concentration of 10 g/15 ml; Lactulose Solution; Mckesson) and 50 g of mannitol (D-mannitol powder; Sigma-Aldrich).

Every morning before data collection, children fasted for at least 2 h, including 1 h of observed fasting, before and after 30 min following the administration of the L:M solution. Children were encouraged to void before administration of the solution and were carefully monitored to ensure that the solution was not spilled, spit out or vomited. In case of any spillage, nurses noted the approximate volume of spillage. Nurses administered a maximum of 20 ml of the solution to each child (2 ml/kg body weight). Both water and breast milk were allowed ad libitum throughout the test after the 2-h mark. Research personnel provided a wholesome lunch for the children ten minutes before the 4-h mark. The urine was collected for a total of 5 h. A sterile adhesive paediatric urine collection bag (PDC Healthcare UR-Assure™ Pediatric Urine Collectors) was placed and changed as needed during a 5-h collection period.

Study nurses collected and measured urine volumes. Thimerosal (Sigma-Aldrich) was added to the urine collection containers to avoid bacterial growth. In the community sites where the collection took place, nurses aliquoted 2 ml of urine for L:M testing, and 50 ml for DON and FB_1 testing.

2.5 | Sample transportation

As soon as blood and urine samples were collected, coded and deidentified, samples were placed in cool boxes filled with icepacks and a thermometer. Samples were transported to the AflaCohort Laboratory situated within the Nepalgunj Medical College, Kohalpur Hospital for processing and storage within 5 h of collection. In the laboratory, the blood samples were allowed to clot at room temperature for half an hour. Samples were then centrifuged at less than 5000 rpm for 10 min. The serum was divided into three aliquots, with one 600 µl aliquot destined for AFB_1 and OTA testing. Blood and urine samples were frozen at −20°C at the local laboratory before being transferred to a −80°C freezer in Kathmandu.

2.6 | Survey and anthropometric data collection at 24–26 months of age

On the day of collection, mothers and children were transported to the closest health facility for data collection. Trained interviewers administered electronic surveys face-to-face in selected community sites (e.g., health centre, local halls, Female Community Health Volunteers' homes). Surveys included qualitative 7 and 24 h food frequency questionnaires with a list of predetermined food items (Campbell et al., 2015).

Upon survey completion, interviewers measured length, weight, head circumference and MUAC to the nearest 0.1 cm and 0.1 kg using ShorrBoard® Measuring Boards, 874 Seca Scales; Seca head circumference measuring tapes and colour-coded paediatric MUAC measuring tapes. Circumference of the upper left arm was measured. Children were excluded from the blood and urine data collection

if they were severely malnourished (weight-for-length [WLZ] z-score ≤ −3, MUAC < 11.5 cm or bilateral pitting oedema).

2.7 | Mycotoxin laboratory analyses

Blood and urine samples from 18- to 22-month-old children were air-shipped on dry ice to the University of Georgia, in the United States, for mycotoxin analysis. Concentrations of mycotoxins were analysed using high-performance liquid chromatography (HPLC) according to methods previously described (Andrews-Trevino et al., 2020; Qian et al., 2013; Wang et al., 1996). Quantification was performed using Agilent 1200 HPLC-fluorescence system. Serum samples were tested for both AFB_1 and OTA. Urine samples from the same visit were tested for DON and fumonisin B1 (UFB_1).

For the AFB_1-lysine adduct, the limit of detection (LOD) was 0.4 pg/mg albumin. The average recovery rate, calculated as the ratio between measured (the mean of three processed samples) and expected (based on standard curve) peak areas of samples spiked with a known amount of standards, was 90% for the report. The AFB_1-lysine adduct concentration was adjusted by albumin concentration, measured via UV/visible spectrophotometry. For serum OTA, the LOD was 0.02 ng/ml and the average recovery was approximately 85%. All sample results were higher than the minimum concentration used for a standard curve, which was 2 pg/injection or 0.02 ng/ml of blood. The LOD for UFB_1 was 0.01 ng/mg creatinine with an average recovery of 83.4%. The LOD for DON was 0.04 ng/mg creatinine with an average recovery of approximately 82.8%.

2.8 | EED laboratory analyses

The L:M ratio is a biomarker commonly used to diagnose EED. A healthy intestine does not absorb lactulose, which is a disaccharide. Mannitol is passively absorbed proportionally to intestinal absorptive capacity. Hence, a larger ratio of lactulose to mannitol indicates intestinal damage, reduced absorptive capacity and increased permeability (Kosek et al., 2014; Morseth et al., 2018; Shulman et al., 1998).

Urine samples (2 ml) from 18- to 22 month-old children were air-shipped on dry ice to the Shulman Laboratory at Baylor College of Medicine in Houston. Samples were analysed for lactulose and mannitol concentrations as described by Shulman et al. (1998) using HPLC methods. The L:M ratio was calculated for children at 18–22 months of age by dividing the urinary lactulose concentration by the urinary mannitol concentration. The lactulose and mannitol excretion ratios were calculated from the measured amount of each in urine (concentration × total urine volume) relative to the initial dose of each sugar.

2.9 | Statistical analysis

Distributions of biomarker values from the 18–22-month visit were assessed for outliers and normality before commencing analyses. Nonnormally distributed data were natural log-transformed for all statistical analyses. Mycotoxin and EED biomarkers were natural log-transformed in all regression models.

Weight and length measurements from the 24–26-month visit were converted to z-scores for weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ) and head circumference (HCZ) with the use of the WHO standards (WHO Multicentre Growth Reference Study Group, 2006). Per the World Health Organization's recommendation for biologically implausible values, z-score outliers (defined as −6 > LAZ > +6, −6 > WAZ > +5, −5 > WLZ > +5, and −5 > HCZ > 5) were excluded before analysis (WHO Multicentre Growth Reference Study Group, 2006). A total of two observations were excluded from the LAZ variable while one observation each was excluded from the WAZ, WLZ and HCZ variables, respectively. Children falling two standard deviations or more below the median of the WHO 2006 Child Growth Standards for LAZ, WAZ, WLZ and HCZ were classified as stunted, underweight, wasted or microcephalic, respectively.

The World Health Organization has established guidelines and indicators to assess infant and young child feeding practices for children aged 6–23 months. The minimum dietary diversity (MDD) indicator is one of eight core indicators (World Health Organization, 2008). MDD is defined as the 'consumption of four or more food groups from the seven food groups for higher dietary quality and to meet daily energy and nutrient requirements of the seven recommended food groups namely: grains, roots and tubers; legumes and nuts; dairy products; flesh foods (meat, fish, poultry and organ meats); eggs; vitamin-A rich fruits and vegetables; other fruits and vegetables' during the previous day. The maternal number of years of education completed was categorised into four groupings: no education, some primary education (1–5 years), some secondary education (6–10 years) and completed secondary or more (>10 years).

Pearson's correlation coefficients were calculated to assess the relationship between mycotoxins levels and L:M measures of EED. Multivariate ordinary least squares regression models with cluster-robust standard errors (Village Development Committees as clusters) were used to assess associations between mycotoxin(s) and length (cm), weight (kg), head circumference (cm), LAZ, WAZ and HCZ. Associations between mycotoxin biomarkers and dichotomous outcomes—stunting, underweight and microcephaly—were assessed using multivariate logistic regression models with cluster-robust standard errors. All models controlled for length, weight or anthropometric z-scores at birth or head circumference at 3 months plus child's MDD (yes/no) and mother's schooling. Covariates in the adjusted models were selected using bivariate analyses, with child length as the dependent variable, and a $p \leq 0.25$.

First, we generated multivariate linear and logistic regression models to assess the association between each individual mycotoxin and each anthropometric outcome with L:M ratio as a covariate (e.g., only AFB_1-lysine adduct as a predictor or only UFB1 as a predictor, see Tables 3 and 4). Second, we constructed models with interaction terms to compute the product of individual mycotoxin exposure and EED (Tables 3 and 4). This was conducted to test for potential interaction effects between individual mycotoxins and EED. Interaction terms statistically significant at the 5% significance level would justify the inclusion of the term

TABLE 1 Descriptive characteristics for subsamples of 699 mother–child dyads in the AflaCohort Study

	Mean ± SD or *n* (%)
Mother	
Age at enrolment (pregnancy), years	23.7 ± 4.7
Education (years)	
None	256 (36.6)
Primary (1–5)	150 (21.5)
Secondary (6–10)	233 (33.3)
More than secondary (>10)	60 (8.6)
Childbirth	
Sex, female	368 (52.7)
LBW < 2500 g	142 (20.6)
Child 18–22 months	
Age (months)	21.3 ± 1.0
Diarrhoea 2 weeks prior	46 (7.0)
Minimum dietary diversity[a]	487 (70.0)
Length (cm)	79.3 ± 3.55
Weight (kg)	9.4 ± 1.2
Head circumference (cm)	45.3 ± 1.4
Length-for-age *z*-score (LAZ)	−1.7 ± 1.1
Weight-for-age *z*-score (WAZ)	−1.6 ± 1.0
Weight-for-length *z*-score (WLZ)	−1.0 ± 0.9
Head circumference *z*-score (HCZ)	−1.4 ± 0.9
Stunted, LAZ < −2 SD	289 (41.5)
Underweight, WAZ < −2 SD	236 (33.9)
Wasted, WLZ < −2 SD	94 (13.5)
Microcephalic, HCZ < −2 SD	169 (24.2)
Child 24–26 months	
Age (months)	25.2 ± 0.8
Length (cm)	81.7 ± 3.7
Weight (kg)	10.0 ± 1.3
Head circumference (cm)	45.6 ± 1.4
Length-for-age *z*-score (LAZ)	−1.8 ± 1.1
Weight-for-age *z*-score (WAZ)	−1.6 ± 1.0
Weight-for-length *z*-score (WLZ)	−0.9 ± 0.9
Head circumference *z*-score (HCZ)	−1.6 ± 0.9
Stunted, LAZ < −2 SD	297 (43.5)
Underweight, WAZ < −2 SD	242 (35.6)
Wasted, WLZ < −2 SD	81 (11.9)
Microcephalic, HCZ < −2 SD	238 (34.9)
Mean follow-up time[b] (months)	4.0 ± 1.0

Abbreviation: LBW, low-birthweight.

[a]Minimum dietary diversity was defined as the proportion of children who received foods made from four or more food groups out of the seven food groups during the previous day.

[b]Mean follow-up time between the 18–22 and 24–26 months visit. Descriptives were limited to children who had participated in the 18–22-month visit in which biomarker data were collected.

in the final models. Third, we generated another series of multivariate linear and logistic regression models, which included all four mycotoxins as predictors for each outcome, with L:M ratio as a covariate to further understand potential combined effects of co-occurring mycotoxins and EED (Table 5). The models also included the aforementioned covariates.

Variance inflation factors helped diagnose multicollinearity among the predictor variables in the regression models. We conducted a sensitivity analysis to assess the robustness of our UFB_1 findings by excluding two outliers with values over 100,000 ng/mg creatinine. Such analyses did not yield divergent results; therefore, these two values were included in the final analyses. All analyses were carried out using Stata 15 software (Stata Corps). A $p \leq 0.05$ was considered statistically different from zero.

3 | RESULTS

3.1 | Demographics

Sociodemographic and anthropometric characteristics of the children and their mothers are presented in Table 1. At enrolment, mothers were on average 24 years old, and approximately 37% of mothers did not have any schooling. Twenty-one percent of children had a low-birthweight (<2500 g). The average length-for-age *z*-score and weight-for-age *z*-scores at the 24–26 months visit were −1.8 ± 1.1 and −1.6 ± 1.0, respectively. Almost 44% of children in the sample were stunted and 36% were underweight at 24–26 months of age. The mean follow-up time between both visits was 4.0 ± 1.0 months.

3.2 | Child mycotoxin and EED biomarkers

Biomarker estimates are presented in Table 2. Eighty-five percent of the children had detectable levels of AFB_1-lysine, with a mean level of 2.41 ± 7.88 pg/mg albumin; 100% had detectable levels of OTA and UFB_1, with a mean of 0.48 ± 1.82 ng/ml and 2594.83 ± 9756.73 pg/mg creatinine, respectively; and 87% of the children had detectable levels of DON, with a mean level of 0.78 ± 5.42 ng/mg creatinine. The average L:M ratio was 0.29 ± 0.53. AFB_1-lysine ($r = 0.0779$, $p < 0.05$) and OTA ($r = 0.1208$, $p < 0.01$) showed significant positive correlations with L:M ratio (Table S1) and OTA had a positive association with L:M ratio after

TABLE 2 Mycotoxin and environmental enteric dysfunction biomarkers in children aged 18–22 months

	n	*n* (%) detectable	Min (>LOD)	Maximum	Average mean (SD)	Geometric mean (CI)
Aflatoxin B1, (pg/mg albumin)	699	595 (85)	0.40	128.07	2.41 (7.88)	1.3 (1.18, 1.36)
Ochratoxin A (ng/ml)	699	699 (100)	0.02	44.49	0.48 (1.82)	0.31 (0.29, 0.33)
Fumonisin B1 (pg/mg creatinine)	683	683 (100)	6.57	132,373.1	2594.83 (9756.73)	192.07 (163.76, 225.28)
Deoxynivalenol (ng/mg creatinine)	689	596 (87)	0.04	129.970	0.78 (5.42)	0.31 (0.28, 0.33)
Total urine (ml)[a]	678	–	10	429	75.09 (63.31)	55.3 (52.1, 58.7)
L:M ratio	675	–	0.02	12.7	0.29 (0.53)	0.22 (0.21, 0.23)
Urinary lactulose (% dose excreted)	675	–	0.004	1.8	0.24 (0.20)	0.17 (0.16, 0.18)
Urinary mannitol (% dose excreted)	675	–	0.02	21.0	5.05 (3.16)	3.94 (3.70, 4.19)
LMER	675	–	0.003	2.5	0.06 (0.11)	0.04 (0.04. 0.05)

Note: LOD: aflatoxin B1 (0.4 pg/mg albumin), ochratoxin A (0.02 ng/ml), fumonisin B1 (0.01 ng/mg creatinine) and deoxynivalenol (0.04 ng/mg creatinine).
Abbreviations: CI, confidence interval; L:M, lactulose:mannitol ratio; LMER, lactulose:mannitol excretion ratio; LOD, limit of detection; SD, standard deviation.
[a]Excludes 21 children with <10 ml of urine.

controlling for other covariates (i.e., other mycotoxins, improved toilet, maternal education and socioeconomic status; Table S2).

3.3 | Associations between individual mycotoxins and EED at 18–22 months of age and continuous child growth outcomes at 24–26 months of age

Table 3 shows the results for regressions examining the association between individual mycotoxins and EED and continuous growth outcomes.

Aflatoxin and EED models: After adjusting for other covariates, AFB_1-lysine levels remained significantly associated with lower weight (*β*: −0.09 kg, 95% confidence interval [CI]: −0.18, −0.002 kg; $p = 0.046$) and lower WAZ (*β*: −0.07, 95% CI: −0.14, −0.007; $p = 0.032$). After adjusting for covariates, L:M ratio remained significantly associated with both lower length (*β*: −0.32 cm, 95% CI: −0.60, −0.03 cm; $p = 0.032$) and weight (*β*: −0.10 kg, 95% CI: −0.20, −0.005 kg; $p = 0.040$).

OTA and EED models: OTA levels were not associated with length, weight or head circumference in the adjusted models. However, L:M ratio was associated with lower length (*β*: −0.34 cm, 95% CI: −0.63, −0.04 cm; $p = 0.030$), lower weight (*β*: −0.11 kg, 95% CI: −0.21, −0.02 kg; $p = 0.019$) and lower WAZ (*β*: −0.08, 95% CI: −0.15, −0.009; $p = 0.029$) in the models.

UFB_1 and EED models: UFB_1 levels were not associated with length, weight or head circumference in the adjusted models. After adjusting for covariates, L:M ratio remained significantly associated with lower length (*β*: −0.37 cm, 95% CI: −0.66, −0.08 cm; $p = 0.015$), lower weight (*β*: −0.12 kg, 95% CI: −0.20, −0.03 kg; $p = 0.011$), lower LAZ (*β*: −0.09, 95% CI: −0.18, −0.001; $p = 0.048$) and lower WAZ (*β*: −0.08, 95% CI: −0.14, −0.02; $p = 0.024$) in the models.

DON and EED models: Neither DON levels nor L:M ratios were associated with length, weight or head circumference in the adjusted models. However, L:M ratio was associated with lower length (*β*: −0.39 cm, 95% CI: −0.68, −0.10 cm; $p = 0.013$), lower weight (*β*: −0.12 kg, 95% CI: −0.21, −0.04 kg; $p = 0.009$), lower LAZ (*β*: −0.09, 95% CI: −0.19, −0.004; $p = 0.041$) and lower WAZ (*β*: −0.08, 95% CI: −0.15, −0.02; $p = 0.018$) in the models.

Regression models with interaction terms: Table S3 shows the results for regressions examining interactions between individual mycotoxins, EED and continuous growth outcomes. None of the interaction terms were statistically significant at the 5% significance level.

3.4 | Associations between individual mycotoxins and EED at 18–22 months of age and dichotomous child growth outcomes at 24–26 months of age

Table 4 shows the results for regressions examining the association between individual mycotoxins and EED and stunting, underweight or microcephaly.

Aflatoxin and EED models: In the individual mycotoxin adjusted models, AFB_1-lysine was associated with increased odds of stunting (OR: 1.28, 95% CI: 1.08, 1.52; $p = 0.004$) and underweight (OR: 1.18, 95% CI: 1.00, 1.38; $p = 0.046$). AFB_1-lysine and L:M ratio were not associated with microcephaly.

OTA, UFB_1, DON and EED models: In the individual mycotoxin models, neither OTA, UFB_1, DON levels nor L:M ratio were individually associated with stunting, underweight or microcephaly.

Regression models with interaction terms: Table S4 shows the results for regressions examining interactions between individual mycotoxins, EED and dichotomous growth outcomes. None of the interaction terms were statistically significant at the 5% significance level.

3.5 | Associations between coexposure to mycotoxins and EED at 18–22 months of age and continuous and dichotomous child growth outcomes at 24–26 months of age

Adjusted regression results for models examining coexposure to mycotoxins, L:M ratio and their association with growth outcomes

TABLE 3 Individual mycotoxin and EED biomarker as predictors of length, weight and head circumference and anthropometric *z*-scores at 24–26 months of age in adjusted linear regression models

	Length (cm) β Coefficient (95% CI)	Weight (kg) β Coefficient (95% CI)	Head circumference (cm) β Coefficient (95% CI)	LAZ β Coefficient (95% CI)	WAZ β Coefficient (95% CI)	HCZ β Coefficient (95% CI)
Aflatoxin B1 (pg/mg albumin)	−0.27	−0.09	−0.07	−0.07	−0.07	−0.03
	(−0.55, 0.02)	(−0.18, −0.002)	(−0.14, 0.0009)	(−0.15, 0.02)	(−0.14, −0.007)	(−0.08, 0.01)
	$p = 0.065$	$p = 0.046$	$p = 0.053$	$p = 0.105$	$p = 0.032$	$p = 0.140$
L:M ratio	−0.32	−0.10	−0.04	−0.07	−0.07	−0.03
	(−0.60, −0.03)	(−0.20, −0.005)	(−0.15, 0.06)	(−0.16, 0.01)	(−0.14, 0.003)	(−0.10, 0.05)
	$p = 0.032$	$p = 0.040$	$p = 0.411$	$p = 0.091$	$p = 0.058$	$p = 0.446$
Ochratoxin A (ng/ml)	0.07	0.07	0.005	0.04	0.06	0.02
	(−0.34, 0.47)	(−0.09, 0.22)	(−0.11, 0.12)	(−0.08, 0.16)	(−0.05, 0.17)	(−0.06, 0.10
	$p = 0.738$	$p = 0.366$	$p = 0.921$	$p = 0.467$	$p = 0.267$	$p = 0.567$
L:M ratio	−0.34	−0.11	−0.05	−0.08	−0.08	−0.03
	(−0.63, −0.04)	(−0.21, −0.02)	(−0.16, 0.06)	(−0.17, 0.01)	(−0.15, −0.009)	(−0.11, 0.05)
	$p = 0.030$	$p = 0.019$	$p = 0.389$	$p = 0.076$	$p = 0.029$	$p = 0.404$
Fumonisin B1 (pg/mg creatinine)	−0.06	−0.02	0.02	−0.02	−0.02	0.009
	(−0.21, 0.08)	(−0.07, 0.03)	(−0.03, 0.07)	(−0.06, 0.02)	(−0.06, 0.02)	(−0.03, 0.04)
	$p = 0.366$	$p = 0.388$	$p = 0.442$	$p = 0.291$	$p = 0.321$	$p = 0.595$
L:M ratio	−0.37	−0.12	−0.04	−0.09	−0.08	−0.02
	(−0.66, −0.08)	(−0.20, −0.03)	(−0.13, 0.05)	(−0.18, −0.001)	(−0.14, −0.02)	(−0.09, 0.04)
	$p = 0.015$	$p = 0.011$	$p = 0.359$	$p = 0.048$	$p = 0.024$	$p = 0.452$
Deoxynivalenol (ng/mg creatinine)	0.06	0.04	−0.03	0.01	0.03	−0.02
	(−0.07, 0.19)	(−0.02, 0.10)	(−0.09, 0.02)	(−0.03, 0.05)	(−0.02, 0.08)	(−0.06, 0.01)
	$p = 0.341$	$p = 0.172$	$p = 0.181$	$p = 0.557$	$p = 0.197$	$p = 0.163$
L:M ratio	−0.39	−0.12	−0.04	−0.09	−0.08	−0.02
	(−0.68, −0.10)	(−0.21, −0.04)	(−0.14, 0.06)	(−0.19, −0.004)	(−0.15, −0.02)	(−0.09, 0.05)
	$p = 0.013$	$p = 0.009$	$p = 0.388$	$p = 0.041$	$p = 0.018$	$p = 0.479$

Abbreviations: CI, confidence interval; L:M, lactulose:mannitol ratio.

TABLE 4 Individual mycotoxin and EED biomarker as predictors of stunting, underweight and low-head circumference at 24–26 months of age in adjusted logistic regression models

	Stunting (%) OR (95% CI)	Underweight (%) OR (95% CI)	Microcephalic (%) OR (95% CI)
Aflatoxin B1 (pg/mg albumin)	1.28	1.18	1.09
	(1.08, 1.52)	(1.00, 1.38)	(0.92, 1.29)
	$p = 0.004$	$p = 0.046$	$p = 0.309$
L:M ratio	1.19	1.01	1.14
	(0.92, 1.54)	(0.77, 1.32)	(0.88, 1.46)
	$p = 0.187$	$p = 0.963$	$p = 0.314$
Ochratoxin A (ng/ml)	1.05	0.92	0.85
	(0.86, 1.29)	(0.72, 1.19)	(0.58, 1.26)
	$p = 0.614$	$p = 0.538$	$p = 0.405$
L:M ratio	1.19	1.02	1.16
	(0.92, 1.54)	(0.78, 1.34)	(0.88, 1.53)
	$p = 0.179$	$p = 0.879$	$p = 0.293$
Fumonisin B1 (pg/mg creatinine)	1.06	1.08	0.95
	(0.94, 1.18)	(0.99, 1.17)	(0.84, 1.09)
	$p = 0.341$	$p = 0.082$	$p = 0.464$
L:M ratio	1.23	1.03	1.14
	(0.96, 1.59)	(0.79, 1.33)	(0.91, 1.44)
	$p = 0.106$	$p = 0.841$	$p = 0.250$
Deoxynivalenol (ng/mg creatinine)	0.99	0.99	1.06
	(0.87, 1.12)	(0.88, 1.10)	(0.96, 1.17)
	$p = 0.845$	$p = 0.805$	$p = 0.278$
L:M ratio	1.23	1.03	1.15
	(0.95, 1.60	(0.79, 1.34)	(0.92, 1.42)
	$p = 0.115$	$p = 0.831$	$p = 0.215$

Note: Cells present ORs, 95% confidence interval, and *p*-value. Covariates: length, weight or anthropometric *z*-scores at birth or head circumference at 3 months, child's minimum dietary diversity (yes/no) and mother's schooling. Due to their skewed distribution, predictors were natural log-transformed before all analyses.

Abbreviations: CI, confidence interval; EED, environmental enteric dysfunction; L:M, lactulose:mannitol ratio; OR, odds ratio.

are presented in Table 5. When adjusting for other mycotoxins, AFB_1-lysine remained negatively associated with child length (β: −0.29 cm, 95% CI: −0.53, −0.05 cm; $p = 0.022$), weight (β: −0.11 kg, 95% CI: −0.18, −0.03 kg; $p = 0.007$), head circumference (β: −0.08 cm, 95% CI: −0.15, −0.004 cm; $p = 0.040$), LAZ (β: −0.08, 95% CI: −0.15, −0.005; $p = 0.038$) and WAZ (β: −0.08, 95% CI: −0.14, −0.03; $p = 0.005$). AFB_1-lysine also remained negatively associated with increased odds of stunting (OR: 1.29, 95% CI: 1.10, 1.50; $p = 0.002$) and underweight (OR: 1.20, 95% CI: 1.03, 1.40; $p = 0.018$). UFB_1 was associated with increased odds of underweight (OR: 1.09, 95% CI: 1.00, 1.18; $p = 0.043$). DON levels were negatively associated with child head circumference (β: −0.05 cm, 95% CI: −0.10, −0.002 cm; $p = 0.044$) and head circumference-for-age *z*-score (β: −0.03, 95% CI: −0.06, −0.0008; $p = 0.045$). L:M ratio was negatively associated with both length (β: −0.33 cm, 95% CI: −0.63, −0.03 cm; $p = 0.031$), weight (β: −0.11 kg, 95% CI: −0.21, −0.02 kg; $p = 0.022$) and WAZ (β: −0.08, 95% CI: −0.15, −0.01; $p = 0.027$).

4 | DISCUSSION

In this study of young children in Banke, Nepal, we examined children's exposure to mycotoxins and its relationship to impaired growth. We also examined if EED is linked to child growth, and if being both exposed to these toxins and having EED is associated with growth impairment. To our knowledge, this is the first study to

TABLE 5 Exposure to multiple mycotoxins and EED biomarkers as predictors of child length, weight, head circumference, anthropometric *z*-scores, stunting, underweight and low-head circumference at 24–26 months of age in adjusted linear and logistic regression models

	Length (cm)	Weight (kg)	Head circumference (cm)	Length-for-age *z*-score	Weight-for-age *z*-score	Head circumference-for-age *z*-score	Stunting (%)	Underweight (%)	Microcephalic (%)
Aflatoxin B1 (pg/mg albumin)	−0.29	−0.11	−0.08	−0.08	−0.08	−0.04	1.29	1.20	1.14
	(−0.53, −0.05)	(−0.18, −0.03)	(−0.15, −0.004)	(−0.15, −0.001)	(−0.14, −0.03)	(−0.09, 0.009)	(1.10, 1.50)	(1.03, 1.40)	(0.96, 1.35)
	$p = 0.022$	$p = 0.007$	$p = 0.040$	$p = 0.038$	$p = 0.005$	$p = 0.099$	$p = 0.002$	$p = 0.018$	$p = 0.134$
Ochratox in A (ng/ml)	0.15	0.10	0.04	0.06	0.08	0.04	0.98	0.88	0.79
	(−0.23, 0.54)	(−0.05, 0.25)	(−0.08, 0.16)	(−0.05, 0.18)	(−0.03, 0.20)	(−0.04, 0.12)	(0.80, 1.19)	(0.67, 1.15)	(0.53, 1.17)
	$p = 0.414$	$p = 0.170$	$p = 0.461$	$p = 0.249$	$p = 0.127$	$p = 0.303$	$p = 0.833$	$p = 0.350$	$p = 0.240$
Fumonisin B1 (pg/mg creatinine)	−0.07	−0.02	0.03	−0.02	−0.02	0.02	1.05	1.09	0.93
	(−0.22, 0.09)	(−0.07, 0.03)	(−0.02, 0.08)	(−0.06, 0.02)	(−0.06, 0.02)	(−0.02, 0.05)	(0.94, 1.18)	(1.00, 1.18)	(0.82, 1.07)
	$p = 0.379$	$p = 0.360$	$p = 0.262$	$p = 0.340$	$p = 0.297$	$p = 0.345$	$p = 0.336$	$p = 0.043$	$p = 0.331$
Deoxynivalenol (ng/mg creatinine)	0.11	0.05	−0.05	0.03	0.04	−0.03	0.95	0.95	1.11
	(−0.03, 0.26)	(−0.02, 0.11)	(−0.10, −0.002)	(−0.02, 0.07)	(−0.02, 0.09)	(−0.06, −0.0008)	(0.84, 1.06)	(0.84, 1.08)	(0.99, 1.24)
	$p = 0.110$	$p = 0.133$	$p = 0.044$	$p = 0.210$	$p = 0.164$	$p = 0.045$	$p = 0.375$	$p = 0.435$	$p = 0.068$
L:M ratio	−0.33	−0.11	−0.04	−0.08	−0.08	−0.03	1.19	1.02	1.14
	(−0.63, −0.03)	(−0.21, −0.02)	(−0.15, 0.07)	(−0.17, 0.009)	(−0.15, −0.01)	(−0.10, 0.05)	(0.92, 1.55)	(0.78, 1.33)	(0.88, 1.49)
	$p = 0.031$	$p = 0.022$	$p = 0.455$	$p = 0.073$	$p = 0.027$	$p = 0.472$	$p = 0.173$	$p = 0.882$	$p = 0.325$

Note: Cells present β coefficient (continuous outcomes) or ORs (dichotomous outcomes), 95% confidence interval, and *p*-value. Linear or logistic regression models included all mycotoxins and L:M ratio. Covariates: length, weight or anthropometric *z*-scores at birth or head circumference at 3 months, child's minimum dietary diversity (yes/no) and mother's schooling. Due to their skewed distribution, predictors were natural log-transformed before all analyses.

Abbreviations: EED, environmental enteric dysfunction; L:M, lactulose:mannitol ratio; OR, odds ratio.

examine coexposure to four common mycotoxins and the first to examine any link between mycotoxin exposure, EED and growth.

In the current study, we found widespread coexistence of multiple mycotoxins in the serum of young children. Specifically, 85% of the sample children had detectable levels of serum AFB_1-lysine adduct at 18–22 months of age, 100% had detectable levels of OTA and UFB_1 and 87% of the children had detectable levels of DON. We also found a simultaneous occurrence of EED in the children; the average L:M ratio was 0.29 ± 0.53. The lack of significance in the interaction terms between mycotoxins and EED suggests the impacts of mycotoxins on growth outcomes do not depend on EED, as measured by L:M ratio. Our results show that while exposure to multiple mycotoxins is common, in multivariate models with all mycotoxins predictors, the only mycotoxin with a consistent relationship to most anthropometric outcomes was aflatoxin; fumonisin had a relationship only with underweight and DON with head circumference.

A recent systematic review by Tesfamariam et al. (2020), concluded uncertainty on whether mycotoxin exposure affects child growth, immunity and mortality while noting the poor quality of evidence available. The latter authors did not rule out a possible association between dietary mycotoxins, including aflatoxin and fumonisin and child malnutrition. A key recommendation from that review was the need to use validated biomarkers of exposure as well as assessment of exposure to multiple mycotoxins and their combined effects on key outcomes of child growth and development. In this study, we were able to quantify exposure to multiple mycotoxins and their combined impact on growth outcomes, as well as study potential interactions between mycotoxins and EED, a suggested pathway to impaired growth.

Few studies have examined exposure to multiple mycotoxins and their association with growth in children and current data are insufficient to assess human exposure and biomonitoring data are scarce. A study conducted in Vietnam that assessed dietary exposure to multiple mycotoxins found a significant negative correlation of each individual mycotoxin (aflatoxin, fumonisin and OTA) and height-for-age *z*-score while only aflatoxin and fumonisin were individually associated with weight-for-height *z*-score (Huong et al., 2019). A study conducted in Tanzania examined fumonisin and DON exposure, in addition to aflatoxin exposure in young children (Chen et al., 2018; Shirima et al., 2015; Srey et al., 2014). While the levels of aflatoxin were not linked with growth impairment, fumonisin exposure was negatively associated with underweight.

In our study, aflatoxin exposure at 18–22 months was linked with growth impairment at 24–26 months, as measured by the child length, weight, head circumference, stunting and underweight, irrespective of exposure to other mycotoxins or EED. We had previously shown an association between aflatoxin exposure and growth using longitudinal AflaCohort Study data (Andrews-Trevino et al., 2021). In this current study, we did not find any significant interaction effects with other mycotoxins or with EED, as noted by the lack of statistical significance of the interaction terms across all models. In other words, while certain mycotoxins have some impact on a range of growth outcomes individually, the impacts do not appear to be dependent on exposure to other risk factors; aflatoxin is the overriding concern as a driver of negative growth outcomes in the context of multiple mycotoxin exposure.

These findings add to the body of evidence hypothesising that aflatoxin can be a significant contributor to poor child growth. Our results support other studies that demonstrate a link between aflatoxin exposure and poor child growth in low-income countries (Andrews-Trevino et al., 2021; Y. Gong et al., 2002; Gong et al., 2004; Turner et al., 2007). That said, our findings differ from two smaller cohort studies conducted in Nepal and Bangladesh, which did not find an association between aflatoxin exposure and growth impairment in the first 36 months of life (Mahfuz et al., 2020; Mitchell et al., 2017). It is possible that the smaller sample sizes in these other studies limited their statistical power to detect differences between AFB_1 and child growth impairment. Our findings also differ from the results of studies in Tanzania (Chen et al., 2018) and Kenya (Hoffmann et al., 2018) showing an absence of an association between aflatoxin exposure and linear growth. The divergent results between children in our cohort study and the aforementioned studies could be due to their smaller sample sizes and/or varying exposure levels as a result of diverse dietary patterns.

We also examined linkages between levels of fumonisin and DON, two other mycotoxins that often contaminate staple foods and both have plausible links to impaired infant growth. Our results showing an inverse link between fumonisin and underweight in the model that controls for exposure to other mycotoxins and EED support findings from the study by Chen et al. (2018) in Tanzania, which found fumonisin exposure was negatively associated with underweight. Children in Tanzania also showed widespread exposure to fumonisins (80% had detectable levels of UFB_1). This is the first study to examine and show a link between DON exposure and head circumference. Based on animal studies, DON is expected to have a negative effect on growth because of decreased food intake and reduced weight gain (Lombard, 2014).

This study has multiple strengths. This is the first study to examine the coexposure to multiple mycotoxins in a large sample of children. It is also one of the first studies to examine potential interactions between mycotoxin exposure and EED, which has been proposed as a possible pathway to impaired child growth. Furthermore, while this analysis was limited to exposure at one time point, the mycotoxin and EED biomarker data preceded the anthropometric measurements, providing unique insight previous cross-sectional studies have not been able to do. Lastly, because the study used a community-based design rather than a clinically based recruitment strategy, results can be generalised to similar populations with similar diets.

A limitation of this study is that we are not able to derive causal relationships due to the nature of the study design in which biomarker data were collected at one point in time and examined alongside child growth a few months later. Secondly, there are numerous mycotoxins detection methods to measure the biomarkers of interest. Layer chromatography with tandem mass spectrometry is one of the gold standards for mycotoxins due to analytical specificity and sensitivity; however, strong HPLC methods, such as those used in this study, are commonly used for several of the discussed biomarkers (Turner & Snyder, 2021). Another limitation was that these data derive from an add-on study to an ongoing birth cohort study, limiting our ability to compute new sample

size and our ability to collect data on children who no longer met the age eligibility requirements. Lastly, while urinary L:M ratio tests are commonly used to identify gut dysfunction, this test has many limitations. Researchers have identified a need for additional field-appropriate biomarkers of EED that can measure different characteristics of EED (Denno et al., 2014; Singh et al., 2021).

In conclusion, our findings suggest that there is an independent association between aflatoxin and linear growth, aflatoxin and fumonisin levels and underweight, and DON and lower head circumference, when adjusting for coexposure of other mycotoxins, EED and other factors. Given these results, we hypothesise that mycotoxin reduction programs, in conjunction with multi-sectoral nutrition interventions, could support improved child growth.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

PW, SG and KB designed the study and contributed to the manuscript. DD, RC, RS, AP and SA provided input into the study design and contributed to the manuscript. DD, AP and SA implemented the study and contributed to the manuscript. J-SW and KX analysed samples and contributed to the manuscript. JA-T designed the research study, analysed the data and wrote the paper. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data described in the manuscript, codebook and analytic code will be made available upon request pending approval. Data will be made publicly and freely available without restriction at https://data.usaid.gov/once all manuscripts related to the study's original research questions have been published in peer-reviewed journals.

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SUPPORTING INFORMATION

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