

A 2-year study reveals implications of feeding management and exposure to mycotoxins on udder health, performance, and fertility in dairy herds

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ABSTRACT

We recently reported the ubiquitous occurrence of mycotoxins and their secondary metabolites in dairy rations and a substantial variation in the feeding management among Austrian dairy farms. The present study aimed to characterize to which extent these factors contribute to the fertility, udder health traits, and performance of dairy herds. During 2019 and 2020, we surveyed 100 dairy farms, visiting each farm 2 times and collecting data and feed samples. Data collection involved information on the main feed ingredients, nutrient composition, and the levels of mycotoxin and other metabolites in the diet. The annual fertility and milk data of the herds were obtained from the national reporting agency. Calving interval was the target criterion for fertility performance, whereas the percentage of primiparous and multiparous cows in the herd with somatic cell counts above 200,000 cells/mL was the criterion for impaired udder health. For each criterion, herds were classified into 3 groups: high/long, mid, and low/short, with the cut-off corresponding to the <25th and >75th percentiles and the rest of the data, respectively. Accordingly, for the calving interval, the cut-offs for the long and short groups were ≥ 400 and ≤ 380 d, for the udder health in primiparous cows were $\geq 20\%$ and $\leq 8\%$ of the herd, and for the udder health in multiparous cows were $\geq 35\%$ and $\leq 20\%$ of the herd, respectively. Quantitative approaches were further performed to define potential risk factors in the herds. The high somatic cell count group had higher dietary expo-

sure to enniatins (2.8 vs. 1.62 mg/cow per d), deoxynivalenol (4.91 vs. 2.3 mg/cow per d), culmorin (9.48 vs. 5.72 mg/cow per d), beauvericin (0.32 vs. 0.18 mg/cow per d), and siccanol (13.3 vs. 5.15 mg/cow per d), and total *Fusarium* metabolites (42.8 vs. 23.2 mg/cow per d) and used more corn silage in the ration (26.9% vs. 17.3% diet DM) compared with the low counterparts. Beauvericin was the most substantial contributing variable among the *Fusarium* metabolites, as indicated by logistic regression and modeling analyses. Logistic analysis indicated that herds with high proportions of cows with milk fat-to-protein ratio >1.5 had an increased odds for a longer calving interval, which was found to be significant for primiparous cows (odds ratio = 5.5, 95% confidence interval = 1.65–21.7). As well, herds with high proportions of multiparous cows showing levels of milk urea nitrogen >30 mg/dL had an increased odds for longer calving intervals (odds ratio = 2.96, 95% confidence interval = 1.22–7.87). In conclusion, the present findings suggest that dietary contamination of *Fusarium* mycotoxins (especially emerging ones), likely due to increased use of corn silage in the diet, seems to be a risk factor for impairing the udder health of primiparous cows. Mismatching dietary energy and protein supply of multiparous cows contributed to reduced herd fertility performance.

Key words: mycotoxins, phytoestrogens, feeding management, dairy cow performance, fertility

INTRODUCTION

Contamination of dairy feed commodities with multiple naturally occurring toxins and endocrine disruptors has been recently emphasized in the literature (Driehuis et al., 2008a; Rodríguez-Blanco et al., 2020; Penagos-

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Tabares et al., 2022a,b). Mycotoxins, to some extent neglected in ruminants (Rodrigues, 2014), are one of the most critical threats to food/feed safety and security due to their effects on human and animal health, making these compounds pose considerable economic implications (Fink-Gremmels, 2008b; Santos and Fink-Gremmels, 2014; Gallo et al., 2015). The toxicological activity of mycotoxins can be manifested as hepatotoxicity, neurotoxicity, nephrotoxicity, and secondary infections induced by immunosuppression, and the signs or lesion manifestations can be belated due to long-term exposure (Sharma, 1993; Fink-Gremmels, 2008b; Sun et al., 2022). Additionally, impaired fertility or disorders could result from dietary exposure to mycotoxins and phytoestrogens. Some fungal metabolites, such as zearalenone (**ZEN**), as well as plant metabolites such as phytoestrogens, are known compounds with estrogenic activity (Reed, 2016; Mostrom and Evans, 2018; Liu and Applegate, 2020). Ruminal microbiota metabolize many toxins (Upadhyaya et al., 2010), including mycotoxins such as ochratoxin A to the less toxic compounds ochratoxin α and phenylalanine (Hult et al., 1976). However, the released metabolites from ruminal mycotoxin degradation are not always less toxic. For instance, the ruminal metabolism of ZEN in the rumen can result in the metabolite α -zearalenol (Hartinger et al., 2023), which has a potency factor of 60 compared with the parent compound (EFSA Panel on Contaminants in the Food Chain et al., 2017). Additionally, other *Fusarium*-derived toxins such as trichothecenes, fumonisins B1 and B2 (**FB1** and **FB2**), deoxynivalenol (**DON**), and beauvericin (**BEA**) have been reported to affect in vitro bovine granulosa cell function, as well as generally affect the reproductive function of domestic animals (Pizzo et al., 2016; Albonico et al., 2017; Chiminelli et al., 2022).

Some of the generic signs associated with subclinical mycotoxicoses in livestock are a reduction of feed intake, a decrease in nutrient absorption, presentation of metabolic disorders, endocrine alterations, and a decline in reproductive as well as productive performance (Richard and Thurston, 1986; Fink-Gremmels, 2008b; Simion, 2018). *Fusarium*-derived mycotoxins and metabolites are considered the most relevant fungal contaminants in the TMR of dairy cows (Nesic et al., 2014; Santos Pereira et al., 2019) and animal feeds, which could be detected across feedstuffs of TMR (Penagos-Tabares et al., 2022a,b). Emerging mycotoxins such as enniatins (**ENN**), BEA, and culmorin (**CUL**) co-occur with other *Fusarium* mycotoxins such as DON, ZEN, and FB1 (Mousavi Khaneghah et al., 2019). Enniatins and BEA have antibacterial and cytotoxic properties that can affect the rumen ecosystem, generating dysbiosis. However, the implications of emerging mycotox-

ins for health and performance in ruminants and other zootechnical species are underexplored (Sy-Cordero et al., 2012; Křížová et al., 2021).

Feeding management affects herds' health, production, and reproduction, as well as the quality and safety of the derived dairy foods (FAO et al., 2014). Feeding management also affects dietary contamination levels with undesirable substances such as mycotoxins. For instance, corn silage is a forage source with high energy and is one of the most common forage ingredients in the diets of dairy and beef cattle in Europe and North America (Wilkinson and Toivonen, 2003). Unfortunately, corn silage is also a primary feed source prone to spoilage and, consequently, a risky feed ingredient concerning mycotoxin contamination (Driehuis et al., 2008b; Penagos-Tabares et al., 2022a,b). Diets fed to dairy cattle without matching their optimal dietary requirements, especially energy and protein, can also lead to health problems, decreased productivity, and fertility (Butler and Smith, 1989; Canfield et al., 1990; Humer et al., 2018).

Monitoring and data collection are essential in assessing dairy herds' performance and health status because they allow the identification of potential for improvement (von Keyserlingk et al., 2012; Egger-Danner et al., 2020). We previously showed that dairy farms varied considerably in terms of feed management, for example, feed choice, nutrient profiles, the hygienic status of feedstuffs, as well as physical characteristics (particle size) of the rations, which had consequences for the dietary contamination of risky naturally occurring compounds (mycotoxins, phytoestrogens, and other secondary metabolites) (Penagos-Tabares et al., 2022b). Exploiting large datasets of cows (provided by the national record agency) from the previously surveyed farms, we explored the risks induced by feeding management parameters and the exposure to mycotoxin/secondary metabolites on the health status, and productive and reproductive performance of Austrian dairy herds. We hypothesized that farms with the greatest mycotoxin contamination levels, high contents of phytoestrogens, and suboptimal nutritional status would have higher risks for impaired health, productive, and reproductive performance.

MATERIALS AND METHODS

Farm Location and Description of Herds

This research was performed in the framework of a large project called "D4Dairy" (<https://d4dairy.com/en/#start>) that surveyed feed safety aspects of the dairy sector in Austria. The data related to 100 dairy farms surveyed, feeds, diets, feeding management as

well as the level of contamination of the feeds with mixtures of mycotoxins and other secondary metabolites are reported earlier (Penagos-Tabares et al., 2022a,b). In brief, for this research, we first identified dairy farms that represent typical dairy production systems in Austria, including farm size, feeding management, and altitudes, and that fulfill these preconditions: (1) the dairy farms are registered with Federal Recording Association (LKV Austria) and provide regular and full records regarding cow performance, breeding, health, and fertility to Zuchtdata EDV-Dienstleistungen GmbH, Austria, (2) have a herd size of >30 lactating cows per farm and operate a dairy freestall, (3) have full documentation of the feeds, pastures, diets, and feeding management including the fertilization and crop rotation for the entire duration of the study, (4) allow full access to their farms for sampling and data collection, as well access to the data as mentioned previously and documentation. From a large pool of farms that fulfilled all these preconditions, we randomly recruited 100 farms for this study. After agreement on data protection and confidentiality with the participating dairy farmers, access to samples and data was provided. Because no animals were involved in this research, Institutional Animal Care and Use Committee approval was not required. The dairy farms recruited were in the 3 provinces leading the country's milk production: Upper Austria ($n = 51$), Lower Austria ($n = 33$), and Styria ($n = 16$). The farms were in zones within altitudes varying from 262 to 1,300 m above sea level. The herd sizes (number of lactating cows according to the data of LKV Austria at the time of investigation according to the record of Zuchtdata EDV-Dienstleistungen GmbH, Austria) were, on average, 67 ± 16 lactating cows per farm, varying from 34 to 152 lactating cows per farm. The annual average of milk production was $8,975 \pm 1,143$ kg/cow per yr (mean $\pm$ SD; range: 6,000–12,000 kg/cow per yr), of fat was 376 ± 46.5 kg/cow per yr (mean $\pm$ SD; range: 250 to 500 kg/cow per yr), and of protein was 316 ± 44.5 kg/cow per yr (mean $\pm$ SD; range: 200–400 kg/cow per yr). The study included computed data from a total population varying from 6,619 to 6,561 lactating dairy cows during the first and second visits. Of this cow population, 28% were primiparous and 73% multiparous. The dominant cattle breed in the farms were Simmental (83.9%) and, less frequently, Holstein-Friesian (8.3%) and Brown-Swiss (4.5%).

Data on Feeding Management and Daily Dietary Exposure to Mycotoxins/Metabolites

Data on feeding management and the estimation of daily dietary exposure to multiple mycotoxins and

other secondary metabolites in 100 dairy farms were obtained based on previously reported data (Penagos-Tabares et al., 2022b). In brief, the multi-metabolite analysis based on a validated multi-metabolite liquid chromatography/electrospray ionization–tandem mass spectrometry (LC/ESI-MS/MS) tested representative ration samples (considering basal feed and concentrate proportions [fed by automatic feeders and robotic milking systems]). Precise information on the sampling method, LC/ESI-MS/MS-based multi-metabolite analytical procedures, and the proximate nutrient analyses is described in Penagos-Tabares et al. (2022b). Determining particle size distribution was done according to Lammers et al. (1996) and Kononoff et al. (2003). The dietary concentrations of mycotoxins, phytoestrogens, and other secondary metabolites are reported in our previous article (Penagos-Tabares et al., 2022b). Estimations of daily exposure (mg/cow per d) to mycotoxins and other secondary metabolites were based on the reported dietary concentrations (expressed as $\mu\text{g/kg}$ DM of ration; Penagos-Tabares et al., 2022b), considering the assumed average of DMI feed data of the study (21.8 kg DM/cow). Information regarding the diet ingredients of the basal feed and DMI was obtained from the personnel responsible for feeding management via interviews guided by a questionnaire. Two visits to each pilot farm were performed. Each visit included feed sampling and an interview concerning feeding management. These visits were carried out from May 2019 to September 2020, with 100 farms during the first round of visits and 98 farms during the second round.

Datasets on the Performance and Health Status of the Herds

Pseudonymized datasets of indicators related to production, fertility, and udder health status of the 100 dairy herds were provided by Zuchtdata EDV-Dienstleistungen GmbH, Austria, within the project. This organization is commissioned to operate the central data processing for the milk recording system and performance testing of Austrian dairy farms. Since 2006 Austria has been implementing a monitoring system of herd health, fertility, and performance, which has become part of the routine performance data recording and is a tool of herd management support for dairy farmers (Egger-Danner et al., 2012; Fuerst and Egger-Danner, 2014). This monitoring system has become widely disseminated; for instance, in 2017, 66% of dairy herds and 80% of dairy cows in the country joined this monitoring system (ZAR, 2018). The collected data include multiple parameters of production (e.g., yield), fertility (e.g., calving interval), health

status (e.g., SCC and diagnostics), and indicators of the nutritional status (such as MUN and milk fat-to-protein ratio [FPR]), among others (Fuerst-Waltl et al., 2016; Egger-Danner et al., 2020). All participating farms achieved milk performance testing approximately every 5 wk. The dataset included information on the number of cows present on the farm during the reference period (one year), mean milk yield (kg), mean calving to first service interval (d), mean calving interval (d), and the nonreturn rate at 90 d (%). All the included herds performed AI by veterinarians or qualified personnel. Udder health variables included mean SCC ($\times 10^3$ cells/mL), percentage of cows in the herd with SCC >200,000, and percentage of cows diagnosed with udder issues (acute mastitis, chronic mastitis, udder edema, and diseases of the udder and the teat) in primiparous and multiparous cows. Milk variables associated with metabolic status indicators such as the rate of cows with milk FPR >1.5 (%) and the incidences of metabolic diagnoses (such as milk fever, clinical ketosis, tetany, other metabolic diseases, rumen acidosis, and displaced abomasum) were also provided. These parameters were derived from validated data previously described by Egger-Danner et al. (2013, 2020).

Statistical Analyses

All previously described data were curated (i.e., organized and integrated) and stored in Microsoft Excel (Microsoft Corp.). All statistical analyses were performed using the procedures of SAS (version 9.4, SAS Institute Inc., Cary, NC). Data on health and performance were at the herd level (i.e., means taken from individual cow data over the year). Subsequently, the herds were classified into 3 groups: low/short, high/long, and mid (medium), corresponding to following the <25th and >75th percentile and the rest of the data, respectively. The duration of the calving interval represented the fertility performance (long: ≥ 400 d, mid: <400 to >380 d, short: ≤ 380 d). The insemination index (defined as the number of inseminations per fertilization) was also considered for the discussion of the results. The udder health was defined by the proportion of cows in the herd that experienced SCC exceeding 200,000 cells/mL milk. The SCC data of primiparous cows (high: $\geq 20\%$ of the herd, mid: >8% to <20%, and low: $\leq 8\%$) were distinguished from multiparous cows (high: $\geq 35\%$, mid: >20% to <35%, and low: $\leq 20\%$). A comparison of these 3 groups per target trait was performed with the MIXED procedure of SAS using the following model:

$$Y = \mu + g_i + r_j + e_{ijk},$$

where μ is the overall mean, g_i is the fixed effect of the group i (high, mid, low), r_j is the random effect of the visit j (1 or 2), and e_{ijk} is the residual error. An orthogonal CONTRAST analysis between high versus low was also performed.

A set of factors (contaminant variables or dietary variables) that detected significant ($P < 0.05$) differences between the groups described above were further tested using quantitative approaches. First, the odds of various factors (i.e., mycotoxin concentrations and dietary factors) to increase the likelihood for herd's long calving interval or high SCC incidence in primiparous and multiparous cows was tested using the logistic approach (PROC LOGISTIC) of SAS. Odds ratio (OR) estimates and the respective profile-likelihood confidence intervals (CI) were computed from exponentiating the difference of the logits between the low versus high groups. The UNITS statement was used to specify units of change for the continuous explanatory variable. Because the explanatory variables differ in value intensities (e.g., high abundant mycotoxins vs. low abundant ones), their standard deviation was used to specify these units of change.

In addition, model building from the same set of target contaminants or dietary variables was done using stepwise logistic regression in PROC LOGISTIC. The final model included only significant predictors ($P < 0.05$) and was accepted when there was no evidence of a lack of fit in the selected model ($P > 0.05$) according to the Hosmer and Lemeshow test. The analysis of maximum likelihood estimates was performed to evaluate the effects of continuous explanatory variables (i.e., Wald chi-square test), and compute the Wald CI and the predicted probability (i.e., using the option PLOTS = EFFECTS). The receiver operating characteristic (ROC) curve and the area under the curve of the models were also computed. The 3-dimensional surface plot underlining the response of the dependent variable (z) to 2 explanatory variables (x and y) was performed using the G3D procedure and the G3GRID procedure of SAS. The G3GRID procedure interpolates the value of the vertical (z) variables for each point on the horizontal (x - y) plane.

RESULTS

Effects on Udder Health Status

As shown in Table 1, dietary concentrations of total fungal metabolites ($P = 0.202$), total *Fusarium* metabolites ($P = 0.006$), DON ($P = 0.015$), ZEN ($P = 0.035$), and the *Fusarium*-derived emerging mycotoxins, including ENN ($P = 0.052$), and BEA ($P = 0.001$), CUL (P

= 0.013), and siccanol ($P = 0.011$), were higher in the high SCC group compared with the low counterparts of primiparous cows. Such differences between the high SCC group versus low SCC were not detected for multiparous cows (Table 1).

Among these potential *Fusarium* metabolites, we showed that BEA was the strongest predictor of the high incidence of primiparous cows showing high SCC counts (>200,000 cells/mL) based on logistic analysis (Figure 1a). Accordingly, the OR was 1.643 (95% CI range: 0.92–3.19). Following the stepwise logistic regression, the final model is $Y = -0.9650 (\pm 0.3709) + [3.3774 (\pm 1.3613) \times \text{BEA (mg/cow per d)}]$, $P = 0.013$. The area under the ROC curve is 0.6264 and high probabilities (>0.6) of herds with high proportions of cows experiencing high SCC levels are predicted when the estimated daily BEA intake >0.5 g/cow (Supplemental Figure S1, <https://doi.org/10.6084/m9.figshare.24657369.v1>; Zebeli, 2023). In contrast, none of the target mycotoxin variables was found to be the significant predictor in multiparous cows. However, DMI was found to be influential. It showed a negative relationship with elevated SCC in multiparous (20.8 kg/cow per d for the high SCC group and 22.5 kg/cow per d for the group with a low SCC group; Supplemental Table S1, <https://doi.org/10.6084/m9.figshare.24657369.v1>; Zebeli, 2023). This was in line with the modeling using the stepwise logistic regression: $Y = 5.5609 (\pm 2.3543) - [0.2520 (\pm 0.1078) \times \text{DMI (kg/cow per d)}]$, $P = 0.019$. The area under the ROC curve is 0.6734 and the model predicted high probabilities (>0.6) of herds with high proportions of multiparous cows showing elevated SCC levels when the DMI is below 20 kg/d (Supplemental Figure S2, <https://doi.org/10.6084/m9.figshare.24657369.v1>; Zebeli, 2023).

Our data further showed that the incorporation of corn silage as well as hay in the diet seems to play a role in udder health, considering that the content of corn silage ($P = 0.002$) and hay ($P = 0.075$) in rations of the high SCC group was higher than the low group (Table 2). Both forage components increased the odds of primiparous cows with SCC exceeding 200,000 cells/mL (Figure 1b). Specifically, the average OR for corn silage was 2.29 (95% CI range: 1.39–4.02, $P = 0.002$), and for hay was 2.50 (95% CI range = 1.08–7.83, $P = 0.08$). In good agreement, the modeling suggested the significance of the corn silage content in increasing the probability of high incidence of elevated SCC in primiparous cows (Supplemental Figure S3, <https://doi.org/10.6084/m9.figshare.24657369.v1>; Zebeli, 2023). The parameter estimates are as follows: $Y = -1.3308 (\pm 0.4612) + [0.0514 (\pm 0.0175) \times \text{corn silage (% in diet DM)}]$, $P = 0.003$, and the area under the ROC curve of the model is 0.6264 (Supplemental Figure S3). More-

over, corn silage contributed to the dietary concentrations of BEA. Figure 2 depicts clearly that herds with primiparous cows with high SCC incidence had both high BEA concentrations and corn silage proportions in their diets.

Concerning multiparous cows, of all *Fusarium* mycotoxins tested, siccanol tended to be greater in the high SCC group compared with the low counterpart, though it did not reach significance ($P = 0.11$; Table 1). Its OR (1.7583; CI = 0.8799–4.0501) for high SCC incidence in multiparous cows was also the highest among other *Fusarium* mycotoxins tested, albeit not significant ($P = 0.137$, Figure 3). Not all mycotoxins negatively affected the udder health of multiparous cows. For instance, the dietary concentration of *Penicillium* metabolites ($P = 0.018$) was lower in the high SCC group than the low SCC group (Table 1).

Effects on Fertility Traits

Data showed that long and short groups did not differ regarding the dietary contamination levels of mycotoxins, mycoestrogens, and phytoestrogens (Table 3). The cut-off was sufficient to identify differences in the other fertility traits, such as resting time and service period, and a higher insemination index than the short counterparts ($P < 0.001$), but neither had any effects on yearly milk yield (Table 4). Although differences in the fertility traits were present, the values suggest that the participating farms in general did not have differences in terms of reproductive performance. In addition, the OR analysis also indicated that whereas DON increased the odds of extended calving interval (OR 1.58, $P = 0.119$), CUL decreased it (0.49, $P = 0.085$; Figure 4).

Our findings confirmed that the nutritional status of dairy cows is critical for fertility, too. Table 2 shows that the NFC content in the diet was higher in the herds with long intervals compared with the short counterpart ($P < 0.05$). The modeling also corroborates such a positive relationship between the dietary NFC content and long calving interval: $Y = -1.8308 (\pm 0.695) + [0.0627 (\pm 0.028) \times \text{NFC (% DM)}]$ ($P = 0.023$) with the area under ROC curve of 0.6389 (Supplemental Figure S4, <https://doi.org/10.6084/m9.figshare.24657369.v1>; Zebeli, 2023). The model predicted high probabilities (>0.6) for herds to have long calving intervals when the dietary NFC content exceeds 35%. Likewise, a significant effect ($P = 0.038$) was detected for the content of particles with a size between 8 to 19 mm (usually associated with cereal grains or grain-containing forages, such as corn silage; Table 2).

Nevertheless, results based on milk component variables of herds with different calving intervals suggest

Table 1. Comparison of the estimated daily dietary exposure (mg/cow per d) to metabolites/mycotoxins in herds differing in udder health status based on the level of SCC in the milk of primiparous and multiparous cows

Metabolite	Based on the incidence of elevated SCC in primiparous cows ^{1,2}						Based on the incidence of elevated SCC in multiparous cows ^{1,3}					
	Low (n = 46)	Mid (n = 144)	High (n = 38)	SE	P	Contrast: high vs. low	Low (n = 36)	Mid (n = 122)	High (n = 40)	SE	P	Contrast: high vs. low
<i>Alternaria</i> -derived	11.52	8.89	9.08	1.92	0.353	0.296	9.32	8.90	9.31	1.86	0.961	0.995
<i>Aspergillus</i> -derived	2.65	2.73	2.84	1.44	0.963	0.784	2.06	2.89	2.17	1.40	0.198	0.874
Ergot alkaloids	0.07	0.20	0.04	0.07	0.147	0.844	0.26	0.08	0.16	0.08	0.136	0.359
<i>Fusarium</i> -derived	23.2	29.0	42.8	7.6	0.018	0.006	27.6	26.9	35.3	6.8	0.327	0.284
<i>Penicillium</i> -derived	4.92	4.12	4.83	0.60	0.393	0.916	5.08	4.33	3.10	0.61	0.052	0.018
Total fungal metabolites	44.6	46.9	62.7	9.41	0.039	0.023	46.1	45.5	52.0	8.63	0.579	0.456
Phytoestrogens	1,718	1,469	1,500	328	0.608	0.493	1,649	1,421	1,386	320	0.64	0.407
Deoxynivalenol	2.30	2.78	4.91	0.65	0.031	0.015	2.89	2.56	4.03	0.64	0.225	0.284
Zearalenone	0.32	0.39	0.67	0.12	0.076	0.035	0.32	0.45	0.35	0.12	0.541	0.825
Fumonisin	2.03	2.23	3.03	0.46	0.368	0.188	2.35	1.98	2.80	0.45	0.379	0.547
Beauvericin	0.18	0.21	0.32	0.03	0.003	0.001	0.18	0.22	0.22	0.03	0.577	0.377
Enniatins	1.62	1.50	2.08	0.26	0.015	0.052	1.51	1.60	1.44	0.24	0.673	0.782
Culmorin	5.72	7.18	9.48	2.00	0.044	0.013	7.59	6.42	7.80	1.85	0.412	0.887
Sicanol	5.15	8.04	13.35	4.13	0.035	0.011	6.21	7.37	11.34	3.74	0.21	0.11
Mycotoxins + phytoestrogens ⁴	1,722	1,472	1,504	328	0.608	0.493	1,653	1,424	1,390	320	0.639	0.406

¹The low, mid, and high groups correspond to the <25th, 25th to 75th, and >75th percentile, respectively.
²Elevated SCC: >200,000 cells/mL; high: ≥20% of herd; mid: >8% to <20% of herd; low: ≤8% of herd.
³Elevated SCC: >200,000 cells/mL; high: ≥20% of herd; mid: >8% to <20% of herd; low: ≤8% of herd.
⁴Mycotoxins = accumulated concentration of zearalenone, alternariol, and alternariol-methyl-ether; phytoestrogens = accumulated concentration of biochanin, coumestrol, daidzein, daidzin, formononetin, genistein, glycitein, glycitin, and ononin.

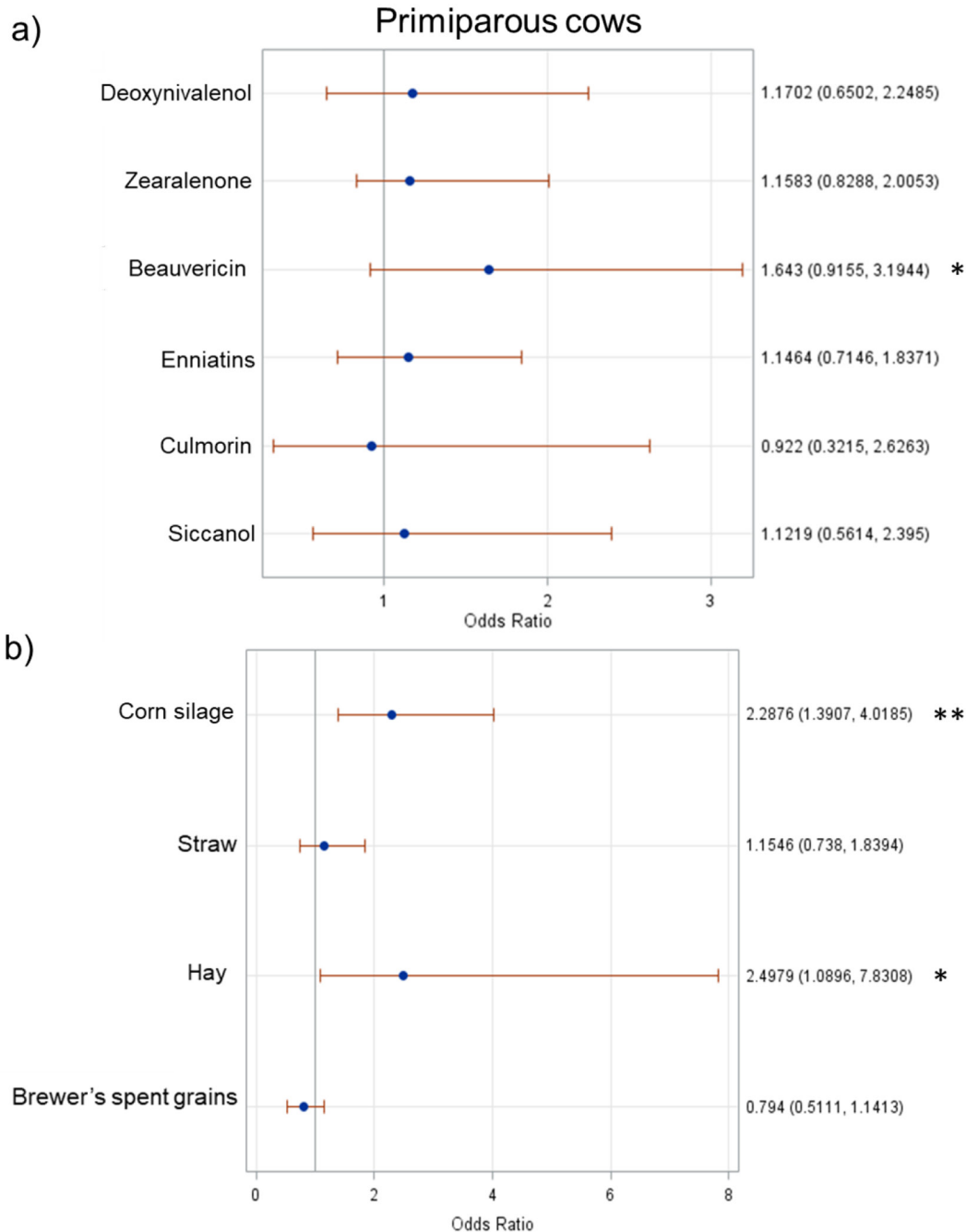


Figure 1. The likelihood of high incidence of udder health (i.e., $\geq 20\%$ of the herd with SCC $> 200,000$ cells/mL) in primiparous cows related to (a) the dietary exposure of *Fusarium*-derived metabolites and (b) the dietary content (% of DM) of corn silage, straw, hay, and brewer's spent grains. Odds ratios estimates with the 95% profile-likelihood confidence limits (values in parentheses). * $0.05 \leq P < 0.15$; ** $P < 0.05$.

that metabolic status may influence the fertility health of the herd (Table 4). For instance, parameters such as MUN and FPR of dairy cows showed negative implications on calving interval. Multiparous cows from herds with longer calving intervals evidenced higher FPR during the 100 d of lactation ($P < 0.001$), a higher occurrence of FPR > 1.5 ($P < 0.001$), and MUN > 30 mg/dL

($P = 0.008$). We observed also that the proportion of cows with high FPR (> 1.5) reflected greater herd variability than when using the mean FPR value that was only 0.02 units apart as a result of high cow-to-cow variation in the herd (Table 4). Primiparous cows from herds with longer calving interval showed a higher incidence of FPR > 1.5 ($P = 0.028$). The herds with longer

Table 2. Comparison of the feeding management parameters in herds differing in udder health status based on the level of SCC in milk of primiparous and multiparous cows

Variable	Based on the incidence of elevated SCC in primiparous cows ^{1,2}						Based on the incidence of elevated SCC in multiparous cows ³					
	Low (n = 46)	Mid (n = 144)	High (n = 38)	SE	P	Contrast: high vs. low ³	Low (n = 36)	Mid (n = 122)	High (n = 40)	SE	P	Contrast: high vs. low
Nutrient composition ⁴												
DM (% as fresh)	36.4	37.2	37.6	0.85	0.435	0.212	36.4	37.4	36.7	0.9	0.472	0.788
CP (% of DM)	15.4	15.4	15.2	0.41	0.827	0.635	15.4	15.4	15.4	0.4	0.989	0.997
Ash (% of DM)	8.5	8.1	8.1	0.25	0.543	0.328	8.2	8.1	8.5	0.3	0.612	0.570
EE (% of DM)	2.8	2.7	2.6	0.07	0.361	0.171	2.7	2.7	2.5	0.1	0.099	0.125
NDF (% of DM)	51.5	50.5	49.0	3.64	0.161	0.057	51.5	50.1	50.6	3.7	0.459	0.518
NFC (% of DM)	21.8 ^b	23.2 ^b	25.1 ^a	3.23	0.075	0.023	22.2	23.7	23	3.3	0.458	0.606
Forage in diet (% of DM)	66	64.9	68.9	1.82	0.106	0.187	68.2	64.6	68	1.9	0.062	0.935
Particle fraction ⁵ (% retained)												
Long	47.3	47.5	43.4	2.61	0.511	0.359	46	46.8	46.7	2.7	0.979	0.886
Medium	23.3	22.5	23.2	1.48	0.885	0.962	25.2	22.6	21.5	1.5	0.301	0.139
Short	25.1	25.5	28.3	1.24	0.217	0.121	24.5	25.9	27.2	1.3	0.451	0.208
Fine	4.3	4.6	5.2	0.38	0.35	0.158	4.2	4.7	4.6	0.4	0.65	0.541
DDMI (kg/cow per d)	21.8	21.7	21.9	0.42	0.941	0.804	22.5 ^a	21.9 ^b	20.8 ^c	0.4	0.033	0.011
Main ingredient (% of DM)												
Corn silage	17.3 ^c	22.9 ^b	26.9 ^a	1.89	0.008	0.002	23.3	21.8	23.3	2	0.773	0.974
Grass silage	46.2 ^a	38.8 ^b	38.1 ^c	2.17	0.022	0.023	41.8	39.9	40.8	2.3	0.815	0.775
Straw	1.9	1.7	1.8	0.29	0.933	0.799	2.4 ^a	1.7 ^b	1.5 ^b	0.3	0.088	0.040
Hay	0.4	0.8	1.7	0.43	0.184	0.075	0.3	0.8	1.5	0.4	0.259	0.108
Brewer's spent grains	1.5	1.0	1.0	0.3	0.459	0.375	1.1	1.2	0.9	0.3	0.790	0.644
Other silages	0.4	0.7	0.8	0.35	0.737	0.451	0.8	0.6	0.7	0.4	0.830	0.831

^a Mean values of feeding management parameters that differ significantly ($P < 0.05$).
¹ The low, mid, and high groups correspond to the <25th, 25th to 75th, and >75th percentile, respectively.
² Elevated SCC: >200,000 cells/mL; high: ≥20% of herd; mid: >8% to <20%; low: ≤8% of herd.
³ Elevated SCC: >200,000 cells/mL; high: ≥35% of herd; mid: >20% to <35% of herd; low: ≤20% of herd.
⁴ Of basal diet. EE = ether extract. The additional fed concentrate was not included.
⁵ Estimated using Penn State Particle Separator consisting of 3 sieves with aperture diameters of 19 mm (long), 8 mm (medium), and 1.18 mm (short) in diameter and pan (fine), on an as-fed basis, according to Lammers et al. (1996) and Kononoff et al. (2003).

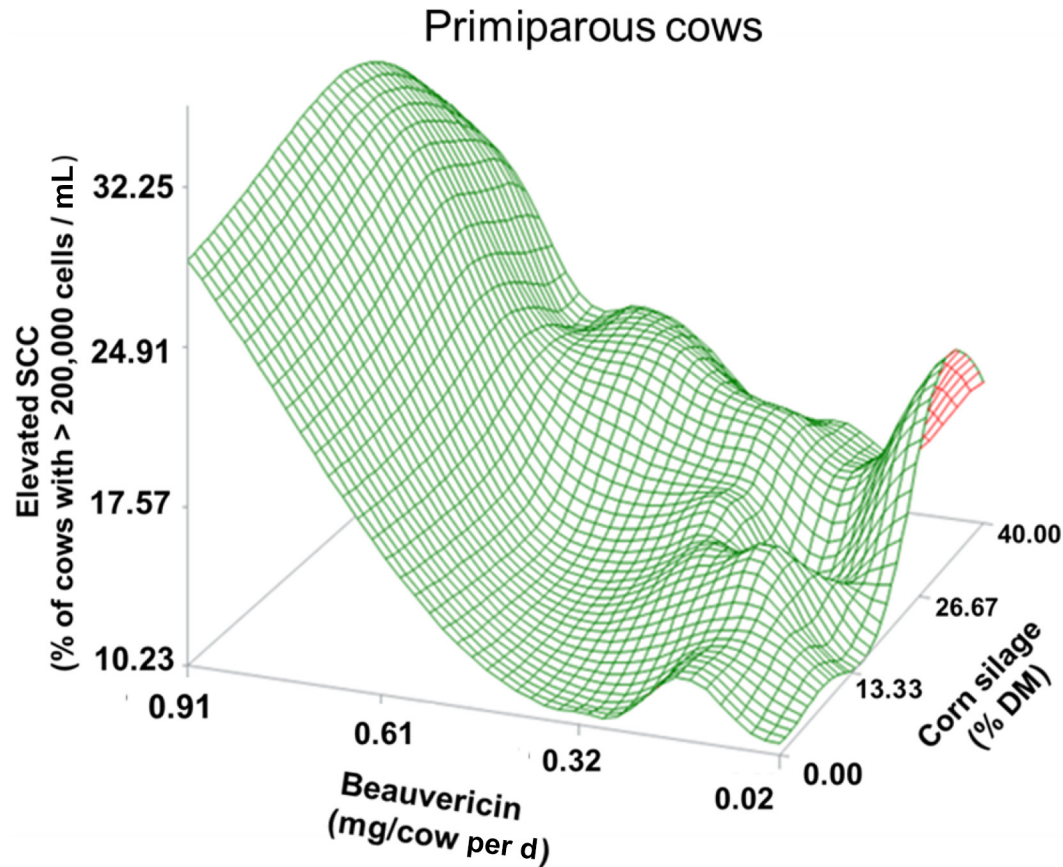


Figure 2. The proportion of primiparous cows in the herd with elevated SCC (>200,000 cells/mL) levels in response to the daily dietary exposure of beauvericin (mg/cow per d) and the dietary content of corn silage (% DM).

calving intervals also had higher incidences of elevated SCC (>200,000/mL) in primiparous ($P = 0.046$) as well as in multiparous cows ($P = 0.005$; Table 4). In addition, logistic analyses were performed to underline the likelihood of long calving intervals with SCC variables (Figure 5a), MUN variables (Figure 5b), and fat-to-protein ratio (Figure 5c). The results confirmed the discrete comparisons that herds with multiparous cows with high MUN (>30 mg/dL) and herds with cows with high FPR (>1.5) had an increased likelihood for long calving intervals. Specifically, the OR for MUN >30 mg/dL in multiparous cows = 2.96 (CI = 1.22–7.87, $P = 0.021$). For milk FPR >1.5, the OR for primiparous cows was 5.49 (CI = 1.65–21.69, $P = 0.009$) and for multiparous cows was 4.08 (CI = 0.98–18.61, $P = 0.058$; Figure 5).

DISCUSSION

The main hypothesis of this research was that farms with the greatest mycotoxin contamination levels would have higher odds for impaired udder health. Using the

SCC as an indicator of udder health, our data indicated that udder health status of primiparous cows was the most responsive variable to dietary contamination of *Fusarium* mycotoxins. In healthy cows, SCC should usually be <200,000 cells/mL, a value generally suggested in the literature to predict intramammary infection (Pantoja et al., 2009; Malik et al., 2018). Previous reports have linked increased SCC and reproductive disruption with the dietary intake of *Fusarium* mycotoxins (Illek et al., 2010; Kiyothong et al., 2012; Falkauskas et al., 2019). However, it is important to note that the dietary levels of contamination with *Fusarium* mycotoxins (DON, ZEN, FB1, and FB2) in our dairy farms were lower than the guidance levels of the European Commission (Penagos-Tabares et al., 2022b). Literature has recognized that low levels of *Fusarium* mycotoxins could have a hidden impact on impairing the udder health status of cows (Fink-Gremmels, 2008b; Gallo et al., 2022), and our data provide evidence linking subtle levels of dietary contamination levels with *Fusarium* mycotoxins with impaired udder health, particularly in primiparous cows. In contrast, our analyses sug-

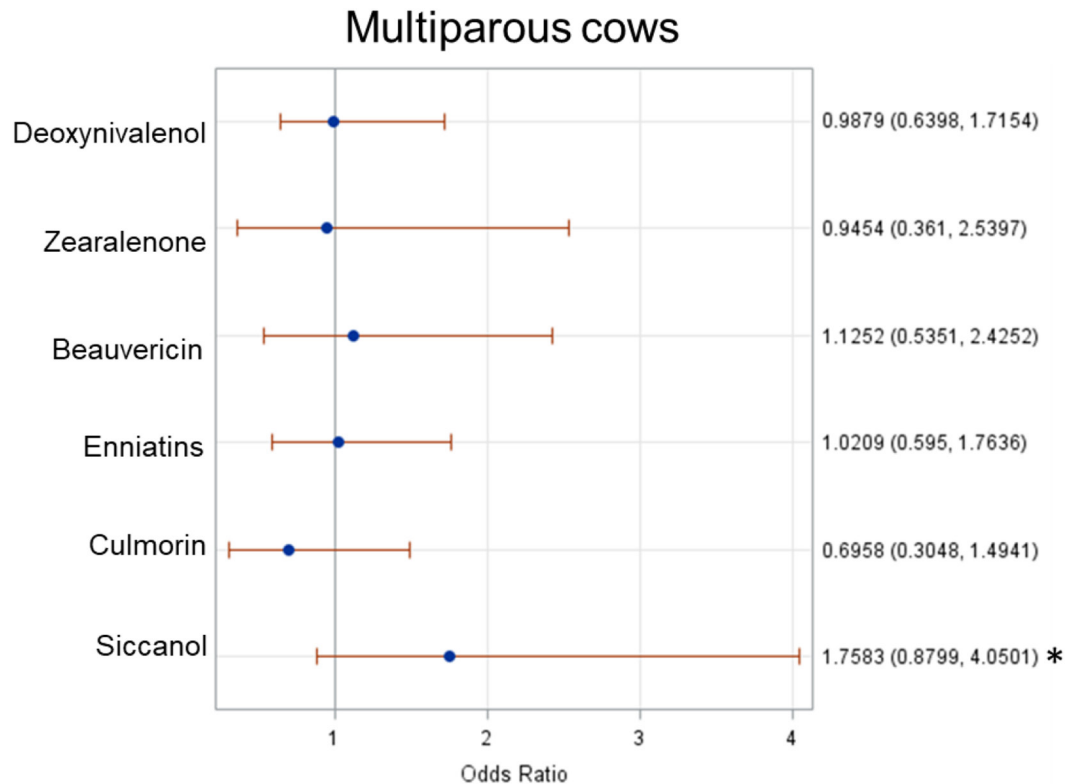


Figure 3. The likelihood of high incidence of udder health (i.e., $\geq 35\%$ of the herd with SCC $> 200,000$ cells/mL) in multiparous cows. Odds ratios estimates with the 95% profile-likelihood confidence limits (values in parentheses). * $0.05 \leq P < 0.15$.

gested that multiparous cows appear more resistant to mycotoxins in terms of udder health than primiparous cows in the herd. The dominant tendency of elevated incidences of herds with elevated SCC incidences under high concentrations of *Fusarium* mycotoxins was more evident in primiparous, suggesting more susceptibility to adverse effects of these kinds of toxins. This can be explained because primiparous cows are generally more predisposed to metabolic and oxidative stress, social stress, rumen acidosis and dysbiosis, adaptation at the physiological level, and negative energetic balance (NEB) than multiparous ones (Yehia et al., 2020; Stauder et al., 2020; Pacífico et al., 2020). It seems that, besides metabolic and general stress related to the periparturient period, mycotoxins can also trigger additional oxidative stress in the organism of mammals (Mavrommatis et al., 2021; Zhang et al., 2021; Fu et al., 2022). In addition, NEB in dairy cattle is directly related to the suppression of the immune system (Goff, 2008; Mordak and Stewart, 2015), and the postpartum primiparous cows show higher susceptibility to NEB impacts than multiparous cows (Yehia et al., 2020).

The results seem to confirm the notion that even low dietary mycotoxin levels can affect the efficiency of dairy herds (Fink-Gremmels, 2008b; Gallo et al., 2022).

Culmorin potentially influences the toxicokinetics of DON due to CUL suppressing the glucuronidation of DON (Woelflingseder et al., 2019). The effect of mycotoxins with antimicrobial activity (i.e., BEA and ENN) in dairy cows could reduce feed conversion and increase the prevalence of diarrheic events, thus decreasing milk production and increasing SCC (Fink-Gremmels, 2008b). Toxic effects of *Fusarium* toxins on the mammary gland have rarely been studied. Recent research on bovine mammary epithelial cells suggests that DON, ENN B, and BEA can impair the bovine mammary gland homeostasis and its innate immune function (Xu et al., 2022), which agrees with and supports our results. Emerging fusarial mycotoxins, such as BEA and ENN, have been defined as fungal toxins that are non-contemplated in the legislation and nonregularly examined but occur commonly in agricultural commodities (Vaclavikova et al., 2013). However, previous studies, including the scientific opinion of the European Food Safety Authority (EFSA, 2014), did not report severe risks for livestock species produced by these emerging mycotoxins (BEA and ENN), highlighting the need for additional toxicological research. Likewise, our results and the high occurrence of these fungal toxins in animal feeds reinforce the idea of the necessity of further

Table 3. Comparison of the estimated daily dietary exposure (mg/cow per d) to metabolites/mycotoxins and other metabolites as well as feeding management parameters in herds differing in fertility performance based on calving intervals

Variable	Based on calving interval ¹					Contrast: short vs. long
	Short (n = 62)	Mid (n = 92)	Long (n = 44)	SE	P	
Metabolite (mg/cow per d)						
<i>Alternaria</i> -derived	7.79	10.72	9.55	1.78	0.249	0.403
<i>Aspergillus</i> -derived	2.78	2.37	3.42	1.42	0.184	0.301
Ergot alkaloids	0.08	0.14	0.22	0.06	0.366	0.157
<i>Fusarium</i> -derived	29.6	27.8	36.4	7.19	0.352	0.294
<i>Penicillium</i> -derived	4.60	4.80	3.47	0.56	0.157	0.139
Total fungal metabolites	47.2	48.2	55.1	9.09	0.486	0.266
Phytoestrogens	1,497	1,684	1,267	323	0.282	0.419
Deoxynivalenol	2.89	2.60	4.32	0.62	0.152	0.139
Zearalenone	0.40	0.35	0.62	0.11	0.137	0.140
Fumonisin	2.40	2.13	2.67	0.45	0.685	0.686
Beauvericin	0.23	0.22	0.23	0.03	0.864	0.870
Enniatins	1.77	1.55	1.64	0.24	0.465	0.566
Culmorin	7.62	6.69	8.05	1.91	0.507	0.755
Siccanol	7.02	8.01	11.11	3.97	0.348	0.158
Mycoestrogens + phytoestrogens ²	1,500	1,688	1,271	323	0.281	0.420
Nutrient composition ³						
DM (% as fresh)	37.6	36.5	37.9	0.8	0.228	0.578
CP (% of DM)	15.4	15.6	14.9	0.4	0.123	0.185
Ash (% of DM)	8.5	8.0	8.1	0.2	0.246	0.292
EE (% of DM)	2.8	2.6	2.7	0.1	0.296	0.605
NDF (% of DM)	51.1	50.8	48.9	3.6	0.154	0.067
NFC (% of DM)	22.2 ^c	23.0 ^b	25.3 ^a	3.2	0.047	0.016
Forage in diet (% of DM)	65.7	65.6	66.9	1.8	0.757	0.533
Particle fraction ⁴ (% retained)						
Long	51.6	44.5	44.2	2.4	0.053	0.053
Medium	20 ^b	24.5 ^a	23.5 ^a	1.4	0.038	0.099
Short	24.2	26.4	27.3	1.2	0.186	0.093
Fine	4.3	4.6	5.0	0.4	0.396	0.396
DMI (kg/cow per d)	22.1	21.6	21.5	0.4	0.491	0.299
Main ingredient (% of DM)						
Corn silage	19.5	23.9	23.4	1.8	0.154	0.167
Grass silage	43.5	38.5	40.2	2.1	0.174	0.308
Straw	1.6	1.8	2.1	0.3	0.512	0.252
Hay	0.9	0.7	1.2	0.4	0.635	0.576
Brewer's spent grains	1.3	1	1.1	0.3	0.763	0.748
Other silages	0.3	1	0.3	0.3	0.192	0.968

^{a-c}Mean values of daily dietary exposures and feeding management parameters that differ significantly ($P < 0.05$).

¹The short, mid, and long groups correspond to the <25th, 25th to 75th, and >75th percentile, respectively. For instance, short: ≤ 380 d, mid: >380 and <400 d, and long: ≥ 400 d.

²Mycoestrogens = accumulated concentration of zearalenone, alternariol, and alternariol-methyl-ether; phytoestrogens = accumulated concentration of biochanin, coumestrol, daidzein, daidzin, formononetin, genistein, genistin, glycitein, glycitin, and ononin.

³Of basal diet. EE = ether extract. The additional fed concentrate was not included.

⁴Estimated using Penn State Particle Separator consisting of 3 sieves with aperture diameters of 19 mm (long), 8 mm (medium), and 1.18 mm (short) in diameter and pan (fine), on an as-fed basis, according to Lammers et al. (1996) and Kononoff et al. (2003).

research on their toxicological properties (EFSA, 2014). The high co-occurrences (>70%) of ENN, BEA, CUL, and others indicate that the research emphasis, risk assessment, and prevention should be focused on these recurrent toxic metabolites and their mixtures (Penagos-Tabares et al., 2022b). These toxins have been neglected in cattle for several years compared with other zootechnical species. Still, now it is well known that the idea that fusarial mycotoxins are only detrimental to

monogastric animals is obsolete and antiquated (Gallo et al., 2022). Mycotoxins can also induce immunotoxic effects (Sun et al., 2022). Interestingly, ENN (A and B) and BEA showed higher cytotoxicity than ZEN and DON on a human neuroblastoma cell line. The same study evidenced that DON possesses more cytotoxicity than the also regulated ZEN (Pérez-Fuentes et al., 2021), suggesting that its toxicity should be seriously considered. In general, in vivo toxic effects of fusarial

Table 4. Comparison parameters of productive and reproductive performance, as well as udder health and metabolic status in herds, with groups differing in calving interval

	Based on calving interval ¹					
Variable	Short (n = 62)	Mid (n = 92)	Long (n = 44)	SE	<i>P</i>	Contrast: short vs. long
Productive performance						
Milk yield (kg/cow per yr)	8.932	9.165	8.638	156	0.039	0.189
Milk fat (kg/cow per yr)	375	381	369	6	0.417	0.539
Milk protein (kg/cow per yr)	314	321	307	6.1	0.192	0.444
Reproductive performance						
Resting time (d)	60.3 ^c	70 ^b	79.5 ^a	1.4	<0.001	<0.001
Service period (d)	83.8 ^c	102 ^b	132 ^a	1.6	<0.001	<0.001
Insemination index (primiparous)	1.90 ^c	2.01 ^b	2.39 ^a	0.07	<0.001	<0.001
Insemination index (multiparous)	1.56 ^c	1.71 ^b	2.09 ^a	0.07	<0.001	<0.001
Cows diagnosed with fertility issues (%)	24.7	23.8	23.4	3.6	0.970	0.816
Udder health status						
Primiparous						
SCC (× 1,000/mL)	120	129	139	6.7	0.176	0.064
Cows with SCC >200,000 cells/mL (%)	12.5 ^b	13.9 ^b	15.4 ^a	0.9	0.133	0.046
Cows diagnosed with udder issues (%)	6.04	5.72	5.00	1.19	0.850	0.573
Multiparous						
SCC (× 1,000/mL)	221 ^c	232 ^b	270 ^a	14.8	0.009	0.003
Cows with SCC >200,000 cells/mL (%)	25.2 ^c	27.0 ^b	30.2 ^a	1.4	0.017	0.005
Cows diagnosed with udder issues (%)	11.6	10.3	13.5	1.5	0.324	0.414
Metabolic status						
Primiparous						
Milk FPR ² the first 100 d of lactation	1.257	1.262	1.274	0.01	0.593	0.315
Cows with milk FPR <1.00 (%)	7.26	7.14	6.92	0.86	0.966	0.795
Cows with milk FPR >1.5 (%)	10.9 ^b	12.4 ^b	15.1 ^a	1.2	0.088	0.028
Cows with MUN >30 mg/dL (%)	8.8	9.5	13.3	1.6	0.144	0.066
Cows with MUN <15 mg/dL (%)	21.3	18.2	19.3	2.2	0.553	0.568
Multiparous						
Milk FPR the first 100 d of lactation	1.23 ^b	1.25 ^c	1.28 ^a	0.008	0.001	<0.001
Cows with milk FPR <1.00 (%)	11.3	10.3	9.6	0.9	0.488	0.241
Cows with milk FPR >1.5 (%)	10.0 ^b	11.9 ^c	16.2 ^a	0.8	<0.001	<0.001
Cows with MUN >30 mg/dL (%)	7.8 ^c	11.2 ^b	13.6 ^a	1.4	0.025	0.008
Cows with MUN <15 mg/dL (%)	20.7	17–0	18.8	2.0	0.326	0.517

^{a-c}Mean values of productive, reproductive performance, udder health, and metabolic status parameters that differ significantly ($P < 0.05$).

¹The short, mid, and long groups correspond to the <25th, 25th to 75th, and >75th percentile, respectively. For instance, short: ≤380 d, mid: >380 and <400 d, and long: ≥400 d.

²FPR = fat-to-protein ratio.

mycotoxins can also be generated by intake reduction and feed refusal, depletion in nutrient absorption, and impaired metabolism, as well as alteration of the endocrine or exocrine systems (Fink-Gremmels, 2008b; Santos and Fink-Gremmels, 2014). Emerging mycotoxins derived from *Fusarium* species have been reported to have detrimental effect on the reproductive performance of domestic animals with different mechanisms, such as impairing the development of oocytes, reducing granulosa cell steroidogenesis, and inhibiting sperm motility, among others (Albonico et al., 2017; Chiminelli et al., 2022). However, more studies on the toxicity and action mechanisms of *Fusarium* mycotoxins (especially the emerging ones) in animals and humans should be addressed (Chiminelli et al., 2022).

The presented results reinforce the idea that corn silage could be a potentially risky feedstuff in the diet of Austrian dairy cows, highly correlated with *Fusarium*

(emerging) mycotoxins. Previous reports also describe corn silage as a potentially risky feed, regarding mycotoxin contamination in dairy cattle (Reisinger et al., 2019; Dänicke et al., 2020). The presented results indicate that higher levels of corn silage can implicate a risk with the increment of SCC, which agrees with an experiment carried out for 12 wk evaluating the effects of diets based on grass silage and corn silage. The grass silage feeding presented decreased SCC compared with their corn silage-fed counterparts (Dänicke et al., 2020). The association between corn silage and disease risks was evidenced via a systematic approach in a study that included 166 Austrian dairy farms with a total of 5,858 dairy cows (Matzhold et al., 2021).

Although a reduction in milk yield performance related to the dietary exposure to mycotoxins was not evidenced in this study, previous research using controlled in vivo experiments has evidenced that *Fusarium*

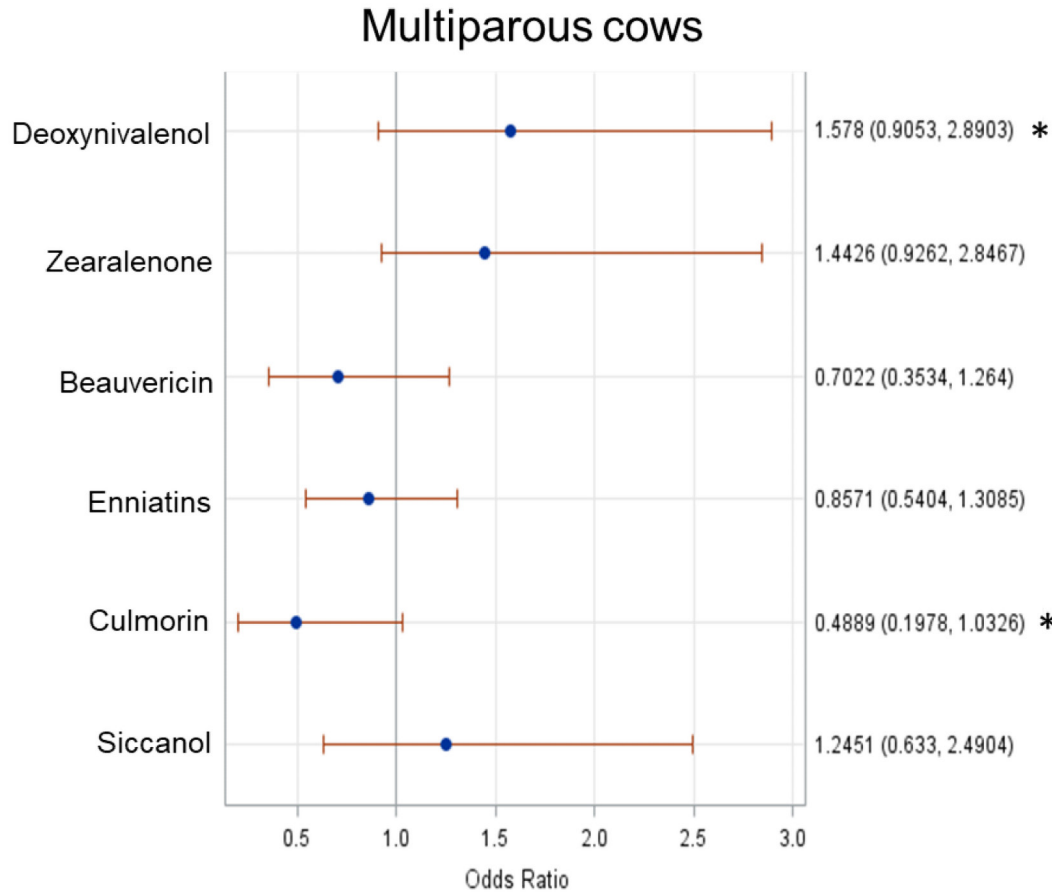


Figure 4. The likelihood of herds with long calving intervals (≥ 400 d) related to the dietary exposure to *Fusarium* mycotoxins. Odds ratios estimates with the 95% profile-likelihood confidence limits (values in parentheses). $*0.05 \leq P < 0.15$.

mycotoxin can reduce milk production (McKay et al., 2019; Falkauskas et al., 2022). An in vivo feeding study with dairy cows during a 28 d period with different concentrations of *Fusarium* mycotoxins (DON and ZEN) below the EU guidance levels can decrease farm productivity (McKay et al., 2019). Another study, which fed diets with 1,966 μg of DON/kg of TMR DM and 366 μg of ZEN/kg of DM did not find an effect on DMI, milk production, or composition of early lactation. Although a not significant decrease of 0.74 kg/cow per d in milk production due to feeding contaminated rations was reported, the reduction in the output generated a significant decline in farm gate income (McKay et al., 2019). Another trial, which fed dairy cows with naturally contaminated TMR with ZEN (300–500 $\mu\text{g}/\text{kg}$) and DON (102–200 $\mu\text{g}/\text{kg}$), evidenced a significantly reduced milk yield (20.8%), fats (11.8%), and proteins (3.5%; Falkauskas et al., 2022). An influence in the hepatic enzymes has been also reported in the same study; for instance, alkaline phosphatase increased by 25%, albumin decreased by 8.7%, and creatinine decreased by 40% (Falkauskas et al., 2022).

Additionally, mycotoxins can modify the rumen microbiota and induce dysbiosis due to their antimicrobial activity and exert a modulating effect on the host animal's immune system, even at low doses (Liew and Mohd-Redzwan, 2018; Hartinger et al., 2022). Mycotoxins represent relevant factors linked to high SCC and udder diseases. In practice, it is not rare to see that the supply of moldy feeds significantly negatively affects udder health and can substantially increase SCC (Fink-Gremmels, 2008a; Kaskous, 2021). Crops and feedstuffs must be protected from contamination with fungi secondary metabolites to avoid the high burdens of these ubiquitous undesirable compounds. When contamination is unavoidable and has been identified, detoxifying substances are promissory approach to reduce the negative impacts of the toxins (Falkauskas et al., 2022). Detoxifying substances (like anti-mycotoxin additives, adsorbents, or binders) can decrease mycotoxin passage to blood and target organs. Previous studies have demonstrated the efficacy of anti-mycotoxin additives in reducing SCC (Kiyothong et al., 2012; Mendoza et al., 2014; Falkauskas et al., 2022).

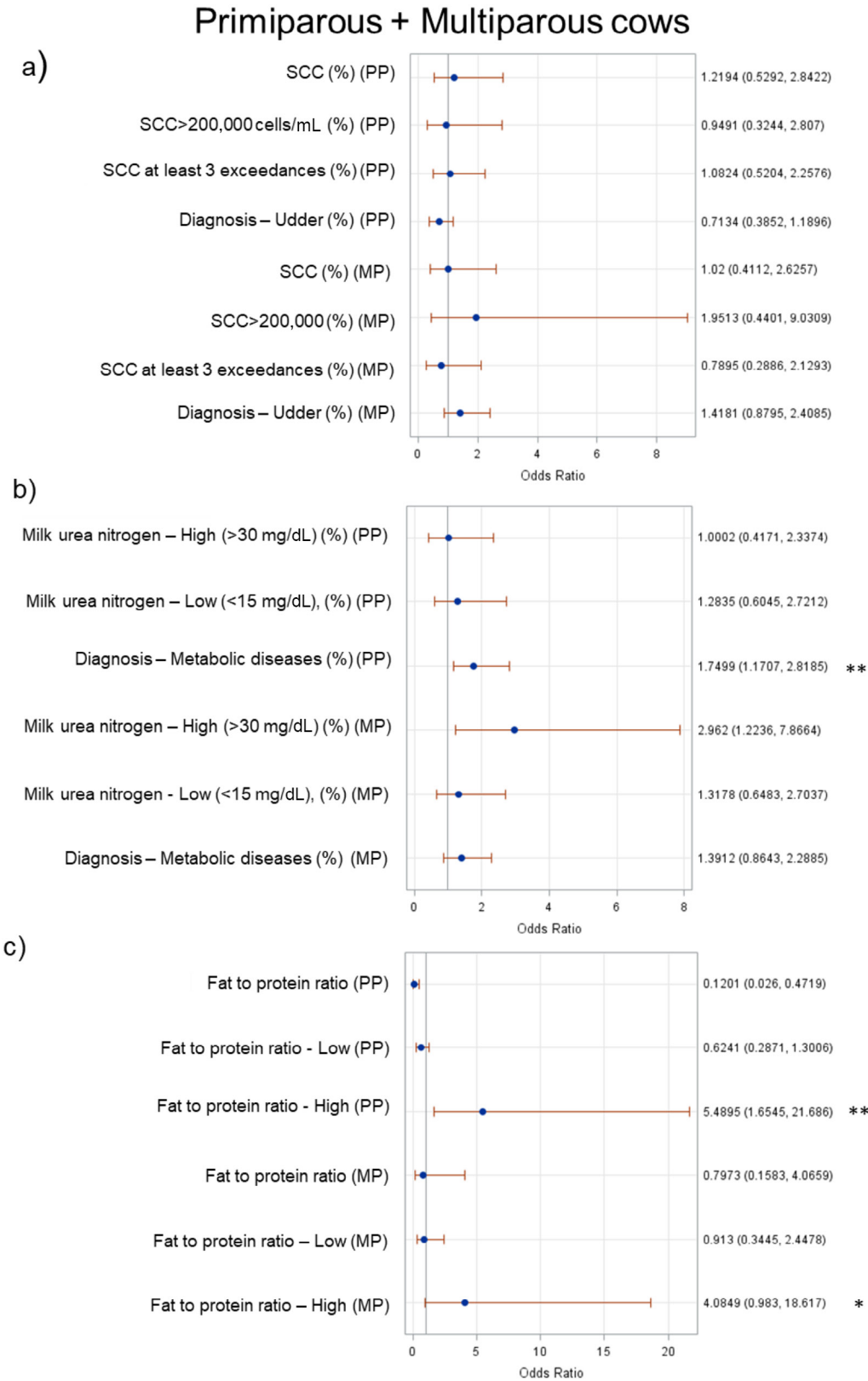


Figure 5. The likelihood of the herd having a long calving interval (≥ 400 d) is associated with (a) SCC-related variables, (b) MUN variables, and (c) milk fat-to-protein ratio values. Odds ratios estimates with the 95% profile-likelihood confidence limits (values in parentheses). PP = primiparous; MP = multiparous. $0.05 \leq P < 0.15$ and $**P < 0.05$.

Regarding the impairment of reproductive performance associated with mycotoxins (also mycoestrogens) and phytoestrogens, our results, with no detected effects, contradicted our expectations. However, it can be explained because the concentrations of the estrogenic compounds (mycoestrogens and phytoestrogens) detected in the surveyed farms were relatively low (Penagos-Tabares et al., 2022b). Although the presented data do not suggest reproductive impairment, several controlled in vivo experiments have evidenced both mycotoxins and phytoestrogens, in higher levels, can impair cow reproductive efficiency (Whitlow and Hagler, 2010; Wocławek-Potocka et al., 2013; Mostrom and Evans, 2018; Grgic et al., 2021). Mycoestrogens and phytoestrogens have shown synergistic estrogenic effects in some in vitro studies (Hessenberger et al., 2017; Vejdovsky et al., 2017). The unnoticeable differences among fertility groups in dietary levels of these potential metabolites might indicate the higher tolerance level regarding reproductive performance in dairy cows. The findings may be related to the farm characteristics. A calving interval of one year is usually considered in dairy farming as the optimum lactation cycle, which includes 10 mo of lactation and 2 mo of dry period (Ma et al., 2022; Temesgen et al., 2022).

Regarding the nutritional status, milk FPR >1.5 is considered an indicator of NEB because lipomobilization increases the fat content, and energy deficiency decreases milk protein (Friggens et al., 2007; Butcher et al., 2012). Cows with FPR >1.5 have been reported showing poor reproductive performance (Heuer et al., 1999). A study criticized that FPR cannot be used as a robust indicator of NEB at an individual cow level (Cabezas-Garcia et al., 2021). Negative energetic balance induces a loss of body tissue mass and is linked with alterations in metabolic and hormone profiles, which in turn influence fertility (Pryce et al., 2001). Milk urea nitrogen is a standard parameter used to estimate diet composition and feeding disorders. Milk urea nitrogen is highly correlated with the BUN, and serves as an indicator of protein intake and nitrogen metabolism in dairy cows (Butler, 1998; Nousiainen et al., 2004). Previous investigations have also suggested the increased levels of MUN are negatively related to dairy cow fertility (Butler, 1998; Rajala-Schultz et al., 2001; Nourozi et al., 2010). In cattle, several interrelated events occurring in various sections of the reproductive tract, resulting in the conception and the establishment of pregnancy, can be influenced by the urea, ammonia, and other toxic products of the protein metabolism, impairing the reproductive efficiency (Ferguson and Chalupa, 1989; Staples et al., 1993; Butler, 1998). Specifically, these physiological key events required until the early pregnancy stage are the progression of

follicular development, the subsequent ovulation, fertilization, transport and development of the embryo(s), maternal-embryonic recognition, and implantation (Leroy et al., 2015; Lonergan et al., 2016). Concentrations of BUN (very correlated with MUN) higher than 19 mg/dL have been linked with altered uterine pH and reduced fertility rate in dairy cows (Butler et al., 1996). Uterine pH has been inversely related to BUN (Elrod et al., 1993; Ocon and Hansen, 2003). The increment of urea not only implicates a pH gradient alteration, but it also increases the secretion of $\text{PGF}_{2\alpha}$ by bovine endometrial epithelial cells (Butler, 1998). Increased uterine luminal $\text{PGF}_{2\alpha}$ triggers luteolysis, interfering with embryo development and survival in cows. This explains another physiological route that links the relationships between elevated MUN, likely as a result of elevated BUN concentrations and decreased fertility performance (Skrzypek et al., 2005; Siatka et al., 2020). As fertility is a multifactorial process that implicates multiple genetic, nutritional, metabolic, and endocrine parameters, results on the relationship between milk production and reproductive performance vary widely between productive systems, farms, and individuals. Previous studies indicate that cows with higher milk production can be positively related to high fertility (López-Gatius et al., 2006; Temesgen et al., 2022). However, extensive literature also suggests that dairy herds with superior milk production generally have poorer fertility (Lean et al., 1989; Butler, 2000; Pryce et al., 2004). Overall, our data suggest that decreased fertility performances can be induced in herds that are oversupplied with protein relative to the energy supply, such that higher CP content in the basal diets would be anticipated, but this was not the case. These results also suggest that the mismatch of nutrients likely originated from the management of additional concentrates given to cows. It is also important to emphasize that the approach of the ecological studies (like presented here) is focused on data at the group (dairy herd) level but not on an individual level (Morgenstern, 1995). Such studies possess practical advantages, and are valid and ideal to generate an initial understanding of the research question. However, these studies also have limitations including methodologic problems that limit causal inference, cross-level bias, as well as problems of confounder control (Morgenstern, 1995). Additionally, data concerning the kinetic and toxicological interaction of mycotoxins and their mixtures are required to understand their metabolism and toxic effects before and after absorption. Some toxic effects can be indirect (not specific against the animal cells), like the disruption of the rumen microbial ecosystem by mycotoxins with antibacterial properties such as the emerging *Fusarium* metabolites BEA and ENN (Sy-Cordero et

al., 2012; Krížová et al., 2021). Rumen dysbiosis can affect the complete physiology of cattle, inducing, among others, udder health issues (Wang et al., 2021) as well as disrupting the rumen metabolism (degradation) of other mycotoxins (May et al., 2000; Debevere et al., 2020). Aspects concerning the toxicokinetic and toxicodynamic of mycotoxins and their mixtures should be explored in the next years to obtain a more holistic overview of the implications of these contaminants on dairy cattle health and performance.

CONCLUSIONS

The findings of this study underline the potential risks of dietary contamination of *Fusarium* metabolites, especially emerging *Fusarium* mycotoxins such as BEA, ENN, and siccanol, on the udder health of dairy cows. Primiparous cows were more susceptible compared with multiparous cows in this regard. Corn silage was found to be a risky feed source affecting udder health traits because it is associated with *Fusarium* mycotoxin contamination in dairy rations. The reported levels of mycotoxins (i.e., mycoestrogens) and phytoestrogens detected in the diets of Austrian herds seem not to affect the reproductive performance greatly. Substandard nutritional status related to the energy and protein of multiparous cows decreased the fertility performance of the herds. Considering the limitations of the ecological studies, research using controlled conditions will be necessary to clarify the effects and thresholds of mycotoxins and other secondary metabolites on the health as well as productive and reproductive performance of dairy cattle. Managing feeding and nutrition of cows will also be instrumental to improve the fertility health of cows in dairy herds.

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