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Health implications and toxicokinetics of short-term graded dietary exposure of Ochratoxin A in dairy cows

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ABSTRACT

Ochratoxin A (OTA) is a nephrotoxic and hepatotoxic mycotoxin commonly found in animal feed, posing health risks to dairy cattle and potential contamination of dairy products. This study examined the effects of short-term (7-day) dietary OTA exposure on dairy cow health and the distribution of OTA and its metabolite ochratoxin α (OTα) across different biological matrices, including plasma, serum, milk, urine, and faeces. Twelve Simmental cows were randomly allocated into two groups receiving either a low (LD, 5 mg/cow/day) or a high (HD, 50 mg/cow/day) OTA dose. Cows were monitored for health parameters including blood chemistry and haematology, chewing, milk and faecal parameters, as well as for the kinetics of OTA from feed to blood, urine, milk and faeces. OTA and OTα were analysed using HPLC-MS. No significant health effects were observed, except for a slight decrease in faecal scores (LD: 2.72 vs. HD: 2.35) and an increase in chewing activity in the HD group (LD: 53.3 vs. HD: 59,9), both within normal ranges. Plasma and serum OTA and OTα levels stabilised after 60 h of exposure, with OTα dominating in faeces and urine, indicating efficient metabolism. OTA was not detected in milk. The results suggest that daily OTA exposure up to 50 mg per cow for seven days does not harm cow health or contaminate milk.

1. Introduction

Ochratoxins are mycotoxins produced by species of the genera such *Aspergillus* (e.g., *A. ochraceus, A. carbonarius*, and *A. niger*), *Fusarium* (e.g., *F graminearum*, *F. verticillioides*, and *F. culmorum*) and *Penicillium* (e.g., *P. verrucosum*), usually found in food and feedstuffs (Kumar et al., 2020). Ochratoxin A (OTA) is the most toxic among the ochratoxins (Heussner and Bingle, 2015). OTA is recognised for its nephrotoxic, hepatotoxic, immunotoxic and teratogenic effects in humans and animals, being categorised as a possible human carcinogen (group 2B) (International Agency for Research on Cancer, 1993). Exposure to OTA can jeopardise livestock health, and the potential transfer of this toxin into the milk, meat and eggs can represent a direct health risk to consumers, thus significantly threatening food safety (Mostrom and Jacobsen, 2020).

Earlier studies have suggested that dairy cattle, like other ruminants, are more resistant to OTA than monogastric species because rumen protozoa and bacteria can deactivate as much as 60 % of the dietary OTA to less toxic compounds such as ochratoxin α (OTα) and L-

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β-phenylalanine (Hult et al., 1976; Kiessling et al., 1984; Mobashar et al., 2010, 2012). In comparison to OTA, the OTα - a dihydroisocoumarin derivative resulting from the cleavage of OTA's peptide bond - is efficiently excreted from the body, thus mitigating the toxicity of OTA exposure (Vettorazzi et al., 2014). However, the toxicokinetics of OTA in cattle also depend on the exposure dose and other factors that regulate the functionality of the gastrointestinal tract (GIT) and its microbiota. For other mycotoxins, such as fumonisin and zearalenone, there is evidence that their intake also jeopardises rumen health and the microbiota in concentrate-rich fed cattle (Hartinger et al., 2022; Rivera-Chacon et al., 2024). Indeed, high-producing dairy cows nowadays are fed diets rich in concentrates to support high milk yields. Concentrate-rich diets are low in physical structure, causing dysbiosis and increasing the passage rate across GIT (Zebeli et al., 2008; Neubauer et al., 2020), which might increase the ruminal washout of OTA and decrease its deactivation through protozoa and bacteria.

Literature findings regarding OTA toxicokinetics in dairy cattle have not been consistent. While Hashimoto et al. (2016) and Zhang et al. (2019) have reported that only minimal OTA levels were detected in the blood of Holstein dairy cows after oral administration, with none found in milk. Other studies have reported the presence of OTA in milk, urine, and organs such as kidneys (Boudra et al., 2007; Keyvan et al., 2018; Pattono et al., 2011; Shreeve et al., 1979; Sreemannarayana et al., 1988). Understanding the extent of OTA transfer from diet into biological matrices such as serum/plasma, milk, urine, and faeces and its impact on dairy cow health is crucial for developing effective mitigation strategies and ensuring dairy product safety. We hypothesised that at a low dietary exposure level, OTA would be largely biotransformed to its less toxic metabolite (OTα), thereby not impairing cow health or being transferred into milk; however, at a high dietary exposure level, the detoxification capacity of dairy cows would be exceeded, leading to detectable systemic exposure in milk. This study aimed to bridge this knowledge gap by assessing the effects of two levels of dietary OTA exposure on the health parameters of dairy cows fed with concentrate-rich diets and by determining the levels of OTA and its metabolite OTα in serum/plasma, milk, urine, and faeces during the exposure.

2. Materials and methods

2.1. Animals, management and experimental design

The experiment and associated animal handling procedures were performed in accordance with the EU Directive 2010/63 for the protection of animals used for scientific purposes, reported according to ARRIVE guidelines, and authorised by the institutional committee of Ethics and Animal Welfare, University of Veterinary Medicine Vienna and the national authority following §26 of the Animal Experiments Act 2012-TVG of the Republic of Austria (GZ: 2022–0.474.452). The experiment was conducted at the research dairy farm "VetFarm" of the University of Veterinary Medicine Vienna in Pottenstein, Lower Austria, for eleven days. Twelve lactating Simmental cows (BW 741.8 ± 75.8 kg; age 5.6 ± 1.9 years; mean ± SD) were housed in a free-stall barn equipped with 12 deep litter cubicles (2.6 × 1.25 m, straw litter) with *ad libitum* access to water and feed prepared as a total mixed ration (TMR). The ration was composed of grass silage, whole-plant maize silage and a commercial concentrate (Milchrinder 18 Kremesberg p, Königshofer GmbH, Austria) at 37.1 %, 12.5 % and 50.4 % on a dry matter (DM) basis, respectively (Table S1). The ration was prepared once daily at approximately 0700 h with an automatic feeding system (Trioliet Triomatic T15, the Netherlands). The TMRs were provided *ad libitum* and offered to the cows twice daily (0800 and 1500 h) in individually assigned automatic single-feed bunks (Insentec B.V., the Netherlands), which were recorded automatically. During the first four days (adaptation phase), all animals were fed the same ration (without OTA) to adapt to the diet and feeders (adaptation phase).

Cows were then randomly assigned to two groups of six animals each and administered one of two OTA doses for seven days. The study size of 12 cows (6 per group) was preferred to balance ethical considerations in line with animal welfare regulations with the need for statistical power, and is consistent with previous toxicokinetic and feeding studies in dairy cattle investigating mycotoxin exposure. OTA was administered using a culture material (KM174, Romer Labs Diagnostic GmbH, Tulln, Austria), which contained 54.8 g of OTA per kg of material Doses of 5 mg and 50 mg OTA/cow/day, corresponding to approximately 250 and 2500 μg/kg on a DM basis, were included in the total mixed ration (TMR). These doses were referred to as Low Dose (LD) and High Dose (HD), respectively, and were based on an estimated feed intake of 20 kg/cow/day, as outlined in guidelines of the European Food Safety Authority (EFSA) (2017). To contextualise these doses within legal limits, the LD level aligns closely with the European Commission's, 2006 guidance, which sets a maximum of 0.25 mg OTA/kg for cereals and cereal products with 12 % moisture used in animal feed. While explicit guidance values exist for pigs, poultry, dogs, and cats, no specific limits are provided for dairy cattle (EFSA, 2023). In the absence of specific thresholds for dairy cattle, the general limit of 250 μg OTA/kg for cereals and cereal products applies (European Commission, 2006). This limit, calculated on an 88 % dry matter basis, corresponds to 284.09 μg/kg at 100 % dry matter. Thus, the doses used in this study (LD and HD) were approximately at and above these guidance levels, respectively. To ensure full dose consumption, OTA was administered in individual feeders mixed into 250 g portions of barley meal (containing either 1.25 mg or 12.5 mg OTA). Four portions were given daily at 8:00, 12:00, 16:00, and 20:00. Following the seven-day experimental period, the cows underwent a 14-day washout phase before their milk was deemed fit for market distribution.

2.2. Feed analyses

Representative samples (2 kg from at least 20 sub-samples) of TMR were collected on day zero (prior to the addition of OTA) and on day seven of the trial and stored at −20 °C until analysis. Once at the laboratory, the samples were dried for 48 h at 65 °C in an air oven and ground through a 0.5 mm sieve (ultra-centrifugal mill ZM 200, Retsch GmbH, Germany) for the nutrient analyses. The latter were carried out according to the Association of German Agricultural Research Centres (Naumann and Bassler, 2012) for DM, ash, organic matter, crude protein, ether extract, neutral detergent fibre, and acid detergent fibre. Particle size distribution of the basal rations was determined in duplicate using a manually operated Penn State Particle Separator (model C24682N, Nasco, Fort Atkinson, WI, USA) with three sieves with aperture diameters of 19 mm, 8 mm, and 1.18 mm, according to Kononoff et al. (2003). The nutrient composition of the ration is shown in Table S1. To avoid toxicological interference from other mycotoxins in the diet, the basal diets were analysed using a validated multi-mycotoxin analysis based on liquid chromatography–tandem mass spectrometry (LC-MS/MS) according to the method described in Penagos et al. (2022). This analysis targeted OTA, aflatoxin B1, deoxynivalenol, zearalenone, fumonisin B1, T-2 toxin and ergot alkaloids (chanoclavine, ergocornine, ergocorninine, ergometrinine and ergovalin). The analysis showed that Aflatoxin B1, zearalenone, T-2 toxin and OTA were not detected, whereas deoxynivalenol, fumonisin B1 and ergot alkaloids were detected at trace levels (17.4, 12.5 and 2.3 μg/kg at 12 % moisture, respectively).

2.3. Collection of animal samples

Blood (for serum and plasma extraction) samples were collected on day 0 (control) and twice per day (morning (at ~0700 h, i.e., before OTA was fed) and afternoon (at ~1600 h) on days 3, 5 and 7 of exposure. Urine samples were collected on days 0 (control), 3, 5 and 7 of exposure in the afternoon (~1600 h). Blood samples were collected from the

jugular vein using serum vacuum tubes (VACUETTE tubes 9 mL CAT Serum Separator Coagulation Activator, Greiner Bio-One GmbH, Austria) and EDTA vacuum tubes (VACUETTE tubes 9 mL K3E K3EDTA, Greiner Bio-One GmbH, Austria). Samples for serum extraction were allowed to clot for one hour at room temperature, then centrifuged for 15 min at 2000 × g and 4°C (centrifuge 5804 R, Eppendorf SE, Germany). For plasma extraction, the samples in EDTA vacuum tubes were kept at 4 °C for a maximum of 2 h and centrifuged for 15 min at 2000 × g at 4 °C. Serum and plasma were transferred into 2 mL tubes (Eppendorf Safe-Lock Tubes 2 mL, Eppendorf SE, Germany) and stored at −20 °C until further analysis. Whole blood samples for haematological analysis were collected in EDTA vacuum tubes and stored at 4 °C in the collection tubes for less than 8 h before laboratory analysis. Milk and faeces samples were collected twice daily during the exposure time (at ~0700 h and after ~1600 h). Milk samples were treated with bronopol (2-bromo-2-nitropropane-1,3-diol) and transported to the regional laboratory of the milk recording organisation (LKV Lower Austria) for analysis, and faeces samples were stored at −20°C until analysis.

2.4. Evaluation of clinical health, chewing activity, and productive performance

Cow health was monitored daily by a veterinarian. The following clinical parameters were determined from day zero to seven twice daily: general behaviour, skin elasticity, internal body temperature (°C), respiratory rate (frequency/min), heart rate (frequency/min), rumen motility (frequency/5 min) (Baumgartner, 2009), rumen fill (1−5) and faecal score (1−5) (Hulsen, 2005). General behaviour was assessed by inspection and described as calm and attentive or slightly to highly reduced or increased. Skin elasticity was assessed by gently pinching and lifting a fold of skin on the lateral mid-neck region, then releasing it and observing the time required for the skin to return to its normal position. A delayed return indicates reduced skin elasticity, which is associated with dehydration. Internal body temperature was measured rectally using a digital thermometer (Microlife VT 1831, Microlife AG, Switzerland). The respiratory rate was determined by counting the number of breaths taken by the animal diagonally behind it on the right side for half a minute. The heart rate was measured by auscultation using a stethoscope (Littmann Classic III stethoscope, 3 M Company, USA) over the left thoracic region at the point of the clearest heartbeat. The rumen was also auscultated in the left paralumbar fossa, and the sounds were counted for a period of five minutes (Hulsen, 2005).

Chewing activity and rumination were continuously assessed throughout the experiment using noseband sensors (RumiWatch System, ITIN + Hoch GmbH, Switzerland) (Kröger et al., 2016). The patterns, the duration of rumination and eating, and the chews per bolus (n/bolus) were synchronised using RumiWatch Manager 2 software (V. 2.2.0.0) and the raw data were processed using the RumiWatch Converter (V. 0.7.3.36.).

2.5. Laboratory analyses

Faeces, blood, and milk samples on day 0 (pre-exposure) and day 7 were analysed for short-chain fatty acids (SCFAs), liver enzymes, haematology, milk composition and somatic cell counts (SCC) in the experimental groups. Blood and serum samples were analysed in the Diagnostic Laboratory of the University of Veterinary Medicine Vienna. Haematological measurements such as red blood cells (RBCs), white blood cells (WBCs), haemoglobin (HGB) and platelets (PLT) were carried out using an automatic analyser ADVIA 2120 (Siemens Healthcare Diagnostics GmbH, Erlangen, Germany). Serum levels of protein, aspartate transaminase (AST), glutamate dehydrogenase (GLDH) and gamma-glutamyltransferase (GGT), as well as albumin and creatine, were measured using a Cobas c501 (Roche Diagnostics, Germany) biochemistry module within 20 min following the pipetting procedure in Cobas e601 (Roche Diagnostics, Germany). Milk composition (concentration of fat, protein, lactose) and SCC were quantified via an infrared analyser (MilkoScan FT-6000, Foss Analytical A/S, Hillerød, Denmark). The faecal concentrations of short-chain fatty acids (SCFAs) such as acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate were determined according to (Qumar et al., 2016) using gas chromatography (GC) with a Shimadzu GC 2010-Plus system (Shimadzu, Japan) and expressed in μmol/g on fresh matter (FM) basis and %. The GC system with a flame ionisation detector and a 30 m × 0.53 mm (inner diameter) × 0.53 μm capillary column (Trace TR Wax, Thermo Fisher Scientific, USA). The identified SCFAs were quantified using an internal standard (4-methylvaleric acid, Sigma-Aldrich®, USA). Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector and detector temperatures were set to 170°C and 220°C, respectively. The generation and evaluation of chromatograms was performed via Stratos Software (Stratos Version 4.5.0.0, Polymer Laboratories, Church Stretton, Shropshire, UK).

2.6. Sample preparation and quantitative analysis of OTA and Otα

Sample preparation was adapted and validated for ruminant matrices from the procedures reported by Streit et al. (2022). Three animals per group were randomly selected for the quantification of OTA and OTα in the different biological matrices. The subgroup size (n = 3 per treatment) was chosen to balance representativeness with the high analytical cost and labour intensity of HPLC-MS/MS, in line with previous toxicokinetic studies in ruminants (e.g. Boudra et al. 2013; Zhang et al. 2019). Creatinine content in urine samples was determined using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS), and samples were diluted individually to 1 mM creatinine with ultrapure water. An aliquot of 200 μL from milk, plasma, serum, or urine (diluted to 1 mM creatinine) samples spiked with a ^{13}C-labelled internal standard of OTA (Romer Labs, Austria) and OTα (produced in-house by enzymatic degradation of ^{13}C-labelled OTA). As extraction solvent, 800 μL of ethyl acetate (HPLC grade from Chem Lab, Belgium) was added, and the samples were put on the vortex mixer for 10 min. After centrifugation (19,000 × g) for 5 min, 400 μL of the supernatant was transferred into a fresh tube and dried under reduced pressure at 45 °C. Residues were reconstituted in 100 μL acetonitrile/water/formic acid (50/49/1 v/v/v). Acetonitrile (HPLC grade) and formic acid were purchased from VWR (Austria). Ultrapure water was produced in-house using a Milli-Q IQ 7000 water purification system (Merck, Germany). Quantification is based on standards in neat solvents. Samples were measured with HPLC-MS/MS according to Streit et al. (2022) using an Agilent 1290 Infinity II coupled to a Sciex QTRAP 6500 + mass spectrometer. Faeces were freeze-dried using VirTis Genesis 35 EL (ATS Scientific Products, USA). Dried faeces (100 mg) were extracted 3 times with acetonitrile/water/formic acid (70/29/1 v/v/v) for 20 min. After each extraction step, samples were centrifuged (3200 × g) for 5 min, and the supernatant was collected. Before measurement, the combined extracts were diluted 1:1.66 in acetonitrile/water/formic acid (50/49/1 v/v/v) and measured using HPLC-MS/MS.

The sample extraction and quantification methods were analytically validated for ruminant biological matrices as described by Streit et al. (2022), including assessments of precision (intra- and inter-day), accuracy, limit of quantification (LOQ), selectivity, and analyte stability in the matrix methods were validated for ruminant matrices as described in Streit et al. (2022), and the following parameters were evaluated: precision (intra-day and inter-day), accuracy, limit of quantification (LOQ), selectivity, and analyte stability in the matrix. Linearity was not re-evaluated as the same parameters and instruments were used, as described in Streit et al. (2022). Accuracy and precision were assessed by spiking blank matrices on two days at different levels of OTA and OTα. The measured concentration was compared to the theoretical spiked concentration in the sample, and the acceptance criteria for all spiking levels was an analyte recovery of 75–125 %. A triplicate was spiked and analysed on the same day to evaluate intra-day precision. For inter-day precision, two independent extractions on different days were assessed.

A relative standard deviation (RSD) < 15 % was set as the acceptance criterion for precision. LOQ was determined using spiking experiments and set to the most negligible concentration where specified accuracy and precision criteria are fulfilled as described in the guidance for industry (Food and Drugs Administration, 2015). In the method validation process, the limit of quantification (LOQ) for Ochratoxin A (OTA) and Ochratoxin α (OTα) was determined across various ruminant matrices, including plasma, serum, urine, milk, and faeces. The LOQ for both OTA and OTα was determined during the method validation at 0.5 ng/mL for plasma, serum, urine, and milk, and 50 ng/g for faeces, based on the performance characteristics and the lower range of the validated analytical method (Table S2). The selectivity of the method was evaluated using the endogenous response of blank samples at the specified retention time of the analytes. An interfering signal of S/N < 3/1 was considered acceptable. Processed sample stability was evaluated at different storage conditions (room temperature, 2–8 °C, or −20 °C) using extracts ready to inject. These samples were measured immediately after sample extraction and after the storage period of 3 days (for RT), 7 days (for 2–8 °C) or 4 weeks (for −20 °C). The acceptance criteria were an analyte recovery of 75–125 % compared to the initial measured concentration.

2.7. Statistical analysis

All data collected were first evaluated using descriptive statistics to determine the variance sizes. The data of health parameters (body temperature, respiratory rate, heart rate, rumen motility, rumen score, faeces scores, blood parameters), milk yield and composition, feed intake and chewing activity and faecal concentration of SCFA were then statistically analysed using SAS (version 9.4; procedure Mixed). The normal distribution of the residuals was checked. A linear model was used for each target group to compare the differences. Measurements taken at different times (day zero and day seven) on the same animal were included as repeated measures in the analysis of variance. The linear model included the day 0 values as a covariate in all analyses. Statistical analyses of the OTA and OTα concentration data (n = 6, 3/group) in biological matrices were performed using various SAS procedures. First, the normality of the data within each dose group was assessed using PROC UNIVARIATE of SAS. The data were deemed normally distributed or acceptable based on the Shapiro-Wilk test ($P > 0.05$) and Q-Q plots. Next, the main effects of dosage and exposure hours were analysed using PROC MIXED of SAS. The statistical model followed the repeated measurements as described by Littell et al. (1998). Accordingly, the model included the dose and time of exposure as fixed effects (main effects), and cow was included as a random effect to account for repeated measures and individual variability. For the urinary concentration, the variance component structure within cows was used instead of autoregressive structure. All values resulting from the mixed model analysis are reported as least square means ± standard error of the mean (SEM). Post hoc pairwise comparisons versus baseline (0 h) were conducted using Tukey-Kramer Test. Significance was declared at $p < 0.05$. In addition, we evaluated individual differences among cows in handling OTA. For that, we modelled for each of the three HD cows the OTA and OTα concentrations in serum, faeces, urine, and milk (only OTα) relative to the time of cumulative exposure to OTA. The data were fitted according to the kinetic model: $y = a + b(1\text{-}e^{-ct})$, where a, b and c are constants fitted, and t is the hour from the first exposure. The modelling was performed using PROC NLIN of SAS. Graphical presentations were done using Microsoft EXCEL.

3. Results

3.1. Effects on health parameters

The administration of OTA at both levels did not significantly affect the clinical parameters, apart from the faecal scores, which decreased in the HD group compared to the LD group ($p < 0.01$). The detailed results and reference values are shown in Table 1. The internal body temperature during the exposure was within the reference values (with averages of 38.40 and 38.41°C for LD and HD, respectively). The respiratory rates of 48.7 and 50.7 (± 3.87) breaths per minute were higher in both groups compared to the reference range. However, it did not differ from the mean value on day 0 (baseline), which was 49.6 breaths per minute (min: 20/min, max: 96/min). General behaviour and skin elasticity were within the normal range in all animals during the study (data not shown).

Regarding blood biochemistry and haematology parameters, no significant differences were detected between the LD and HD groups (Table 2). Concentrations of creatinine, total protein, albumin, AST, GLDH, and GGT were within physiological reference ranges in all animals. Haematological parameters, including haemoglobin, haematocrit, erythrocyte indices, and leukocyte profiles, also remained within normal ranges. Differential leukocyte counts and proportions of segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils showed no values outside the expected physiological limits

3.2. Effects on milk yield and composition

During the seven-day exposure period, milk yield of cows appeared unaffected by OTA exposure levels. (Table 3). Milk components, including fat, protein, fat-to-protein ratio, and lactose, did not show detectable differences between the LD and HD groups and were generally within physiological reference ranges. A slightly lower milk fat content and fat-to-protein ratio were observed in the HD group; however, these minor deviations should be interpreted cautiously given the limited sample size. The determination of the milk SCC indicated values well below the threshold value of subclinical mastitis (200.000/mL) (Malik et al., 2018; Pantoja et al., 2009) in both groups.

3.3. Effects on feeding behaviour, chewing activity and SCFA faecal concentration

During the dietary exposure period, feed intake and chewing activity of cows were generally similar between groups. A difference was detected in chewing activity per minute (Table 4), but no other feeding behaviour parameters showed clear group differences. Average feed intake was 22.3 kg DM/d in the LD group and 22.9 kg DM/d in the HD group, with a slight increase in both groups compared to baseline (LD: 19.8 kg DM/d; HD: 20.3 kg DM/d). Cows spent 164.8 min/d (LD) and 157.1 min/d (HD) eating, which was comparable to baseline values (mean 160.9 min/d) These findings should be interpreted with caution due to the small sample size. The animals of the LD group ruminated 6 chews less per minute than their HD counterparts (95 % CI 2.1–9.9; $P < 0.05$), but the number of chews per bolus was not different between the two groups (Table 4). The drinking behaviour was analysed based on the daily drinking time and the number of sips, which was unremarkable. The composition of SCFAs in faeces showed no significant differences in the total amount and most of the individual SCFAs (except for valerate, $p < 0.0001$) between the LD and HD groups (Table 5).

3.4. Concentrations of OTA and OTα in different biological matrices

Methods for quantifying OTA and OTα in ruminant plasma, serum, urine (diluted to 1 mM creatinine), milk, and faeces were validated, and validation results are summarised in Table S2. Method accuracy, over all matrices and tested spiking levels, was between 78 % and 123 %. The maximum intraday precision with RSD 10.5 % and the maximum interday precision with RSD 14.1 % fulfilled RSD acceptance criteria of < 15 %. Processed sample stability was evaluated at different storage conditions (RT for 3 days, 2–8 °C for 7 days, and −20 °C for 4 weeks). The analyte recovery after storage was between 89 % and 117 % from the initial measured concentration for all conditions tested. Selectivity

Table 1
Clinical health parameters of lactating Simmental cows (n = 12, 6 per group) measured after seven days of exposure to low (5 mg/cow/day) and high (50 mg/cow/day) dietary ochratoxin A (OTA) intake.

Parameters	OTA-Low	OTA-High	SEM	p-value	Reference value	References
Body temperature (°C)	38.4	38.4	0.11	0.94	38.3–38.8	Baumgartner, 2009
Respiratory rate (Frequency/min)	48.7	50.7	3.87	0.72	10–30	
Heart rate (Frequency/min)	77.5	76.3	1.77	0.64	60–80	
Rumen motility (Frequency/5 min)	6.04	6.27	0.20	0.45	5–10	
Rumen fill score (1−5)	3.5	3.4	0.12	0.54	3	Hulsen, 2005
Faeces score (1−5)	2.7	2.4	0.14	< 0.01	3	

SEM: standard error of the mean.

Table 2
Blood chemistry and haematology of lactating Simmental cows (n = 12, 6 per group) after seven days of low (5 mg/cow/day) and high (50 mg/cow/day) ochratoxin A (OTA) intake.

Parameters	OTA-Low	OTA-High	SEM	p-value	Reference value	Reference
Creatinine (mg/dL)	1.02	1.01	0.02	0.63	1–2	Kraft and Dürr, 2013
Total protein (g/dL)	7.18	7.13	0.14	0.84	6–8	Douglas and Wardrop, 2010
Albumin (g/dL)	4.01	3.95	0.02	0.52	-	-
AST (U/L)	85.3	90.0	4.49	0.48	43–127	Kraft and Dürr, 2013
GLDH (U/L)	21.1	16.9	5.01	0.58	< 30	
GGT (U/L)	31.5	30.2	3.03	0.76	15–39	
Erythrocytes (10^6/μL)	5.6	5.9	0.13	0.21	4.9–7.5	Douglas and Wardrop, 2010
Haemoglobin (g/dL)	9.8	10.1	0.21	0.26	8.4–12.0	
Haematocrit (%)	25.6	26.8	0.51	0.14	21–30	
MCV (fL)	45.7	45.9	0.20	0.58	36–50	
MCH (pg)	17.5	17.4	0.08	0.46	14–19	
MCHC (g/dL)	38.2	37.9	0.16	0.26	36–39	George et al., 2010
Leukocytes (/μL)	6619.2	6580.8	337.7	0.94	5100–13.300	Douglas and Wardrop, 2010
CHCM (g/dL)	37.1	36.9	0.096	0.14	-	-
MPXI	6.04	5.71	0.62	0.72	-	-
RDW (%)	16.8	16.3	0.30	0.28	16–20	Douglas and Wardrop, 2010
Segment nuclei (/μL)	2809	2753	263.1	0.89	1700–6000	
Lymphocytes (/μL)	2637	2812	74.7	0.19	1800–8100	
Monocytes (/μL)	361	430	38.3	0.24	100–700	
Eosinophils (/μL)	644	537	87.3	0.41	100–1200	
Basophils (/μL)	55	64	4.6	0.19	0–200	
Large unstained cells/ unclassifiable cells (/μL)	52	56	21.4	0.91	-	-

SEM: standard error of the mean; AST: Aspartate-Amino transferase; GLDH: Glutamate dehydrogenase; GGT: Gamma-glutamyl transferase; MCV: mean red cell volume; MCH: Mean haemoglobin content of the individual erythrocytes; MCHC: Mean haemoglobin concentration of the erythrocytes; CHCM: Mean cellular haemoglobin concentration; MPXI: mean myeloperoxidase index; RDW: Red blood cell distribution width.

Table 3
Milk yield and milk composition of lactating Simmental cows (n = 12, 6 per group) after seven days of low (5 mg/cow/day) and high (50 mg/cow/day) ochratoxin A (OTA) intake.

Parameter	OTA-Low	OTA-High	SEM	p-value	Reference value	Reference
Milk yield (kg/d)	31.8	33.1	0.73	0.23	-	-
Fat (%)	3.61	3.42	0.22	0.56	3.5–4.5	George et al., 2010
Protein (%)	3.39	3.43	0.06	0.64	3.2–3.8	Kraft and Dürr, 2013
Lactose (%)	4.65	4.73	0.04	0.14	4.6–5.0	
Somatic cell count (10^3/mL)	84.6	59.9	11.41	0.16	< 200	
Urea N (mg/dL)	16.8	15.9	1.62	0.72	15–30	
pH	6.56	6.52	0.03	0.43	6.4–6.6	
Fat: protein ratio	1.11	0.98	0.08	0.29	1.0–1.4	

SEM: standard error of the mean.

was assessed with the endogenous response of the blank matrix. No interfering peaks at the retention time of the analytes were visible. Therefore, the described methods were considered fit for their intended purpose and were applied to the samples.

The observed concentrations of OTA and OTα in plasma, serum, urine, faeces, and milk between the LD and HD groups are shown in Figs. 1–3 and in supplementary Tables S3-S7. Increases were observed at multiple time points; however, statistical significance relative to baseline (0 h) was reached only when the magnitude of change exceeded the detection threshold of the statistical model. Specifically, higher OTA and OTα concentrations were detected in all these matrices in the HD group compared to the LD group. Fig. 1 illustrates the time-dependent concentrations of OTA and its metabolite OTα in the plasma (Fig. 1A and Table S3) and serum (Fig. 1B and Table S4) of dairy cows administered LD and HD of OTA. In the HD group, OTA concentrations in plasma showed an increase during the initial exposure period, reached a peak at approximately 60 h, and then fluctuated over time. The highest plasmatic concentration of OTα was detected at 156 h of exposure. OTA and OTα concentrations in plasma and serum were consistently higher in the HD group compared to the LD group throughout the exposure period ($p < 0.05$). The LD group, on the other hand, maintained consistently low OTA concentrations in plasma throughout the exposure period. Similarly, OTα serum concentrations in the HD group demonstrated a significant rise with the highest peak at 156 h (24.8 ng/mL). The LD group maintained low OTα levels, lower than 2 ng/mL, throughout the exposure period (Tables S3 and S4).

Fig. 2 and 3 presents the time-dependent concentrations of OTA and its metabolite OTα in the urine (A) and faeces (B). In faeces, OTA concentrations in the HD group exceeded 130 ng/g DM after 36 h and showed visible fluctuations during the exposure period, with higher

Table 4
Comparison of the effects of low (5 mg/cow/day) and high (50 mg/cow/day) intake of ochratoxin A (OTA)feed intake and chewing activity of lactating Simmental cows(n = 12, 6 per group) during seven days of exposure.

Parameter	OTA-Low	OTA-High	SEM	p-value	Previous reported values[1]	Reference
Feed intake (kg DM/d)	22.3	22.9	0.56	0.51	-	-
Eating time (min/d)	164	157	21.7	0.80	211–319	Braun et al., 2015
Ruminating time (min/d)	500	518	17.9	0.50	370–511	Braun et al., 2015
Drinking time (min/d)	10.0	9.6	1.77	0.87	-	-
Total chewing activity (n/d)	41,127	41,383	1004.8	0.86	-	-
Sips (n/d)	183	163	28.8	0.61	-	-
Bolus (n/d)	488.	448.	34.3	0.42	347–478	Braun et al., 2015
Ruminating activity (chews/min)	53.3	59.9	1.9	0.04	-	-
Ruminating activity (chews/bolus)	45.0	48.4	2.1	0.27	44.3–69.4	Braun et al., 2015

[1]Values of the chewing activity reported in the literature. SEM: standard error of the mean.

Table 5
Concentration and proportions of short-chain fatty acids (SCFA) in faeces of lactating Simmental cows (n = 12, 6 per group) fed either low (5 mg/cow/day) andhigh (HD: 50 mg/cow/day) dosages of ochratoxin A (OTA) after seven days of dietary exposure.

Parameter	OTA-Low	OTA-High	SEM	p-value	Reference value	Reference
Total SCFA (μMol/g)	52. 3	46.7	2.27	0.29	-	Neubauer et al., 2020
Acetate (%)	74.1	74.5	0.53	0.57	75.5 ± 0.77	
Propionate (%)	18.2	17.9	0.37	0.65	14.3 ± 0.29	
Butyrate (%)	4.84	4.86	0.21	0.97	4.3 ± 0.17	
Isobutyrate (%)	1.16	0.96	0.13	0.41	3.9 ± 0.24	
Isovalerate (%)	0.73	0.40	0.98	0.07	0.84 ± 0.047	
Valerate (%)	1.09	1.20	0.28	< 0.01	1.14 ± 0.05	

SEM: standard error of the mean.

concentrations observed around 108 h and 156 h (Table S5). Faecal OTα concentrations also peaked in the HD group at 108 h reaching concentration of 1670 ng/g DM. From hours 36–156, OTα concentrations remained over 1100 ng/g of DM. The LD group showed no OTA detection and low OTα concentrations in faeces across the exposure duration, with a maximum of 153.7 ng/g DM at 84 h (Tables S5). For the HD group, OTA concentrations in urine showed significantly higher levels over the exposure period than 0 h. The substantial increase was detected at around 34 μg/mmol creatinine at 60 h, which remained elevated at 108 and 156 h. OTα concentrations in urine followed a similar trend, peaking at approximately 2100 μg/mmol creatinine at 60 h after the first exposure, with average levels over 1750 μg/mmol creatinine noted at 108 and 156 h. In contrast, the LD group exhibited consistently low OTA and OTα concentrations in urine throughout the exposure period. In the LD group, at only 156 h, the urinary concentrations of OTA and OTα were elevated but did not reach significance compared with the 0 h (Table S6).

Generally, OTA was not detected in any milk sample, but OTα was detected (Fig. 3, Table S7). In the HD group, OTα concentrations in milk increased during the exposure period, with the highest observed values of approximately 4.44 ng/mL at 48 h and, and 4.8 ng/mL at 144 h. Statistically significant differences compared to baseline (0 h) were only detected at 48 h, 60 h 132 h, 144 h and 156 h (Table S7). The LD group maintained consistently low OTα concentrations in milk throughout the exposure period, with only a minor increase at 144 h, reaching approximately 1.7 ng/mL. Although this concentration was above baseline, it was not statistically significant in compared with 0 h (Table S7). Within the HD group, while all cows maintained relatively similar concentrations of OTA and OTα in the blood samples, they seemed to vary somewhat in their ability to metabolise and excrete the measured metabolites (Figure S1).

4. Discussion

This study evaluated the effects of OTA challenge on dairy cows' health parameters and performance during a seven-day administration

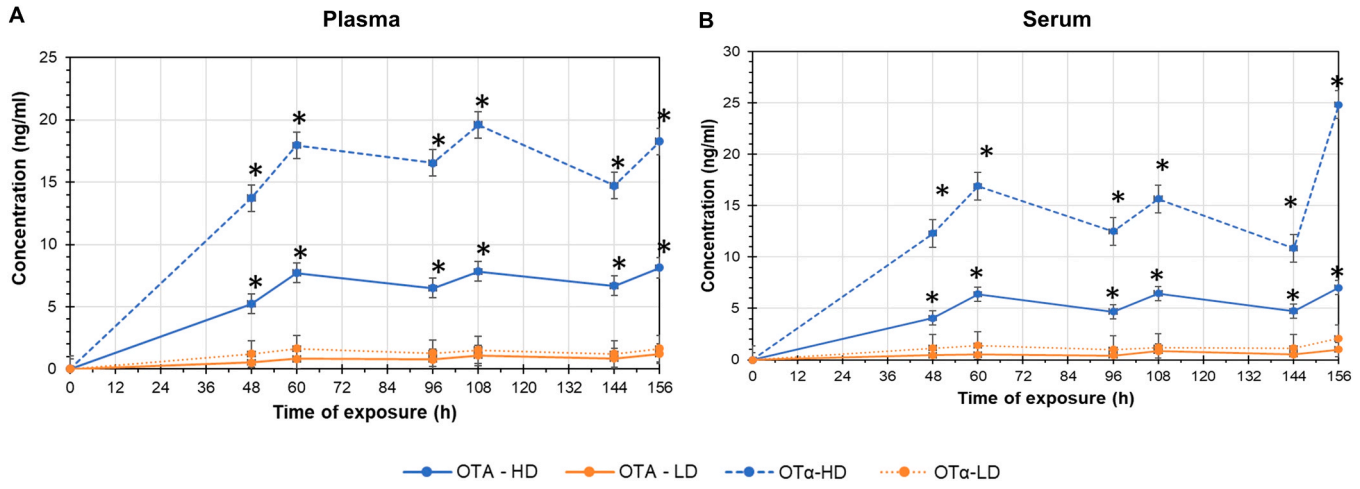

Fig. 1. Kinetics of ochratoxin A (OTA) and ochratoxin α (OTα) in (A) plasma and (B) serum of lactating Simmental cows continuously exposed for seven days to low (LD: 5 mg/cow/day) or high dosages (HD: 50 mg/cow/day) of OTA. * Indicates significant differences of the respective dosages and metabolites compared with 0 h of exposure (baseline) ($P < 0.05$) according to Tukey-Kramer test. Error bars are pooled SEM (See Tables S3 and S4, with levels of OTA and OTα in plasma and serum, respectively). (n = 6 cows, 3 per group).

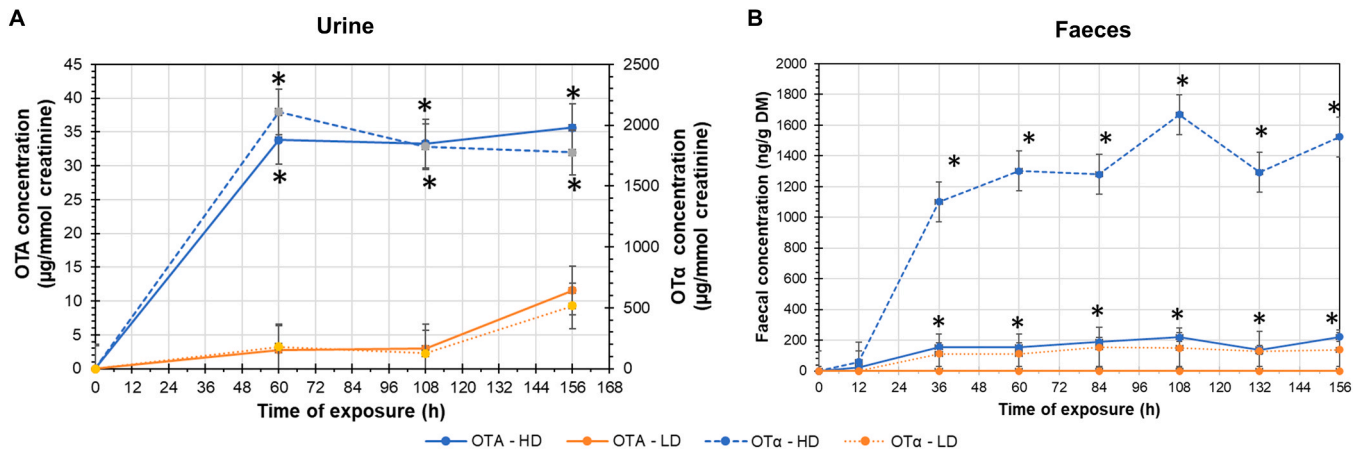

Fig. 2. Kinetics of excreted concentrations of ochratoxin A (OTA) and ochratoxin α (OTα) in (A) urine and (B) faeces of lactating Simmental cows continuously exposed for seven days to either low (LD: 5 mg/cow/day) or high dosages (HD: 50 mg/cow/day) of OTA. * Indicates significant differences of the respective dosages and metabolites compared with 0 h of exposure (baseline) ($P < 0.05$) according to the Tukey-Kramer test. Error bars are pooled SEM. (See Tables S5 and S6, with levels of OTA and OTα in urine and faeces, respectively). (n = 6 cows, 3 per group).

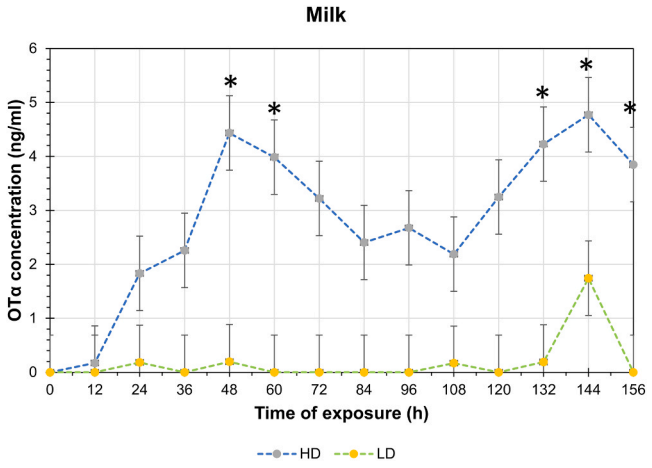

Fig. 3. Kinetics of ochratoxin α (OTα) in milk of Simmental cows exposed continuously for seven days to either low (LD: 5 mg/cow/day) or high ochratoxin A (OTA) dosages (HD: 50 mg/cow/day). * Indicates significant differences in the respective dosages and metabolites compared with 0 h of exposure (baseline) ($P < 0.05$) according to the Tukey-Kramer test. Error bars are pooled SEM. (See Table S7). (n = 6 cows, 3 per group).

of OTA at doses of 5 mg and 50 mg per cow per day. This study was conducted using Austrian-bred Simmental dairy cows, which represent the predominant dairy breed in Austria (Ledinek et al., 2019). The use of this breed is relevant because Simmental cattle are commonly used in commercial dairy production in Europe (Shevhuzhev et al., 2017), and their metabolic and production characteristics may influence OTA kinetics. The high milk yield of the herd reflects typical production levels for this breed, supporting the applicability of our findings to standard dairy farming conditions. We observed only limited non-clinically important changes in the health of either dose group during the 7 days of the study. In the case of severe, acute intoxication with OTA, fed as a single dose of 13.3 mg OTA/kg BW, other authors have reported loss of appetite, cessation of milk production, difficulties in standing up and diarrhoea (Ribelin et al., 1978). In our study, the LD is equivalent to approximately the EU-guidance value (0,25 mg/kg 88 % DM), while the HD group received a dose ten times above this level (European Commission, 2006). Considering the average BW of the cows involved in this study, the LD group received approximately 0.0067 mg OTA/kg BW and the HD group approximately 0.0674 mg OTA/kg BW, corresponding to about 1985- and 197-fold lower doses than those reported by Ribelin et al. (1978), respectively. The feed intake also remained undisturbed. Few parameters of chewing activity deviated from previously reported values, but it can be assumed that these may depend on several factors, such as the ration composition, the physiological stage, performance and breed. No reduction in milk yield or milk composition was detected either, consistent with the study by Hashimoto et al. (2016), in which cows were exposed to OTA at dietary concentrations of 5, 50 or 100 µg/kg DM corresponding to approximately 0.16, 1.6 and 3.2 µg OTA/kg BW/day, or 0.10, 1.0 and 2.0 mg OTA/cow/day for 28 days, respectively, based on the reported average daily feed intake of 20 kg and body weight of 620 kg (Hashimoto et al., 2016). Faecal consistency scores remained within the normal physiological range throughout the exposure period. None of the cows exhibited a score of 1, which would indicate intoxication or rumen acidosis. Most animals had scores of 2 or 3, with similar distributions between the LD and HD groups. Another plausible factor influencing faecal consistency is the shift in SCFA composition, particularly the relative increase in valerate observed in the HD group compared with the LD group. Branched-chain SCFAs (isoacids), such as isobutyrate and isovalerate, are established markers of ruminal protein fermentation, produced through the deamination and decarboxylation of branched-chain amino acids (valine, leucine, and isoleucine) by rumen microbes (Lui et al., 2019). An increase in valerate may therefore indicate an enhanced contribution of proteolytic fermentation processes in response to OTA exposure. Since SCFAs, including branched-chain isoacids, play key physiological roles in ruminants, such as regulating water and electrolyte absorption, rumen motility, and epithelial metabolism (Firkins et al., 2024). Even modest alterations in SCFA profiles can influence osmotic balance and gut motility, thereby modifying faecal texture without necessarily producing overt clinical signs such as diarrhoea. Although the changes observed in VFA composition were limited, they may reflect mild perturbations in ruminal microbial activity induced by OTA exposure, potentially contributing to subtle differences in faecal consistency.

However, the minor changes in the concentrations of SCFAs found in this study due to HD can be considered as biologically irrelevant to be able affecting faecal consistency.

An important aspect of OTA toxicokinetics in ruminants is the amount of OTA the rumen can degrade. A functional rumen is crucial for the degradation of OTA to the non-toxic OTα and L-β-phenylalanine and, thus, for higher tolerance to mycotoxin (Battacone et al., 2010). Two *in vivo* experiments compared OTA toxicokinetics in pre-ruminant and functional ruminant calves (Xiao et al., 1991a,b). In the first experiment, milk-fed (preruminant) calves were administered 1.0 or 4.0 mg/kg of

OTA BW and died within 24 h, regardless of the dose. In the second experiment, functional ruminant calves fed a mixed diet of barley and hay showed no adverse effects when given 2.0 mg/kg of OTA BW. The rumen's ability to hydrolyse OTA has been demonstrated *in vitro* using cow rumen fluid (Hult et al., 1976; Müller et al., 1998; Müller et al., 2001). However, there is evidence that the rumen microbiome does not hydrolyse all of OTA; thus, some OTA is absorbed (Hashimoto et al., 2016). The amount not subject to microbial degradation is estimated at 5–62 %, and although no obvious clinical pathologies occur, the amounts are systemically present and detectable, for example, in plasma (Hashimoto et al., 2016). In the latter study, cows were dosed with either 5, 50 or 100 μg OTA/kg feed (equivalent to approximately 0.16, 1.6 and 3.2 μg OTA/kg BW/day, or 0.10, 1.0 and 2.0 mg OTA/cow/day) for 28 days, and the circulating OTA values exceeding the LOD of 0.1 μg/kg were detected on some days, especially in 100 μg group. However, significant differences were not found (Hashimoto et al., 2016). In the current trial, the doses of 5 and 50 mg OTA per cow per day were many times higher than in the previously described study, but OTA was administered for only one week. With this short time frame, our data suggest that the rumen can effectively biodegrade an amount of 5 mg OTA daily (i.e., LD group) and that very low concentrations of OTA were present in the circulation and no OTA was quantified (LOQ: 0.5 ng/mL) in milk from these cows. However, the rumen seemed to reach its capacity to handle 50 mg OTA daily with elevated concentrations of circulating OTA, occurring only after 48 h from the first exposure. This may suggest an accumulative effect of OTA exposure. The elimination time of OTA from the rumen could be extended depending on the dosage as well as the diet (Mobashar et al., 2010, 2012). In the rumen, protozoa are the main microbes responsible for OTA degradation (Özpinar et al., 1999). Multiple dietary factors, such as concentrate proportion and feed particle length, affect rumen microbiota, including protozoa (Zebeli et al., 2008) and can therefore influence the ruminal degradation of OTA. Although we did not measure the protozoal population in our study, we may assume that our 50 %-concentrate diet, which is a typical dairy ration, provided the microbes with sufficient energy in the form of starch as well as with optimal particle size, preventing a rapid washout from the rumen. In supporting this assumption, Müller et al. (1998) also found that the rate of OTA degradation increased when grass silage was replaced with hay or grass, and when the concentrate content increased from 10 % to 50 %. Özpinar et al. (1999) also found that the rate of OTA degradation increased with higher numbers of rumen protozoa and diet starch concentration, suggesting that optimizing nutrient availability of microbiota can enhance OTA degradation.

Our data also showed that the OTA bypassing ruminal biodegradation and its metabolite OTα are absorbed across gut epithelia, entering into the bloodstream, so that OTA and OTα increased in plasma and serum with increasing OTA dosage. Because OTA is strongly protein-bound, we quantified OTA and OTα in both matrices (plasma and serum) to account for potential matrix effects; concentrations in the two matrices followed the same dose-dependent trend, with only minor, non-systematic differences (Tables S3–S4). Measuring OTA and OTα in plasma is commonly preferred for kinetic studies because it reflects the circulating concentration at the time of sampling (Hashimoto et al., 2015; Zhang et al., 2019). However, serum can also provide reliable quantification, particularly in routine biomonitoring, as the difference in OTA partitioning between plasma and serum is minimal in ruminants. While most animal species OTA is absorbed from the stomach (Gupta, 2007), in ruminants the small intestine is the major OTA absorption site (Mobashar et al., 2010). OTA is distributed to various organs with the kidneys being the main site (Gupta, 2007). Our data suggest that major routes of OTA excretion are urine and faeces. The efficiency of this translocation process is contingent on several factors, including the functionality of the rumen and the dietary composition. Studies have demonstrated that OTA is absorbed relatively quickly in ruminants with a functional rumen, showing a bimodal serum concentration-time curve indicative of initial rapid absorption followed by secondary peaks due to enterohepatic recirculation (Xiao et al., 1991 a,b). OTA circulates from the liver into bile and into the intestine, where it is then metabolised to OTα (Gupta, 2007). This absorption and subsequent recirculation suggest a complex interplay between the digestive and circulatory systems in managing OTA levels in the body.

An interesting finding of this study was that OTA not detect in the milk, whereas y its non-toxic metabolite OTα was consistently measurable particularly in the HD group. Other *in vivo* studies have shown low or no transfer of OTA to milk (Hashimoto et al., 2016; Zhang et al., 2019), whereas multiple studies evidenced milk contamination with OTA. For example, in France, OTA was detected in 1.1 % (3/264) of analysed raw bulk milk sample at low levels ranging from 5 to 8 ng/L (Boudra et al., 2007). In a Chinese study, OTA was detected in 25.8 % (31/120) of pasteurised milk samples, with a maximum detection of 18.8 μg/kg (Zhang et al., 2022). Shreeve et al. (1979) reported that no OTA residues were detected in the muscle, liver, kidney, serum or milk of cows fed with diets containing 0.317–1.125 mg of OTA/kg for 11 weeks. Hashimoto et al. (2016) reported that OTA was not detected in milk or edible tissues, including muscle, fat, and liver, following 28 days of feeding at lower doses (e.g., 5, 50, and 100 μg OTA/kg DM) compared with the doses used in our study. Indeed, from the results of this study, OTA appears not to be carried over into the milk of cows when dietary concentrations of OTA are within the range of 250 and 2500 μg/kg DM. Such discrepancies likely reflect differences between controlled experimental conditions and real-world settings. In experimental studies, feed is homogenous, and contamination levels are precisely controlled, allowing for efficient ruminal biodegradation of OTA and resulting in minimal systemic transfer. By contrast, in field conditions, chronic low-level exposure, variable feed quality, multiple contamination sources, and differences in rumen health or function may increase OTA carryover into milk. Additionally, analytical detection limits and sampling strategies can also contribute to differences in reported OTA occurrence in milk. The studies on OTA ingestion in cows reported thus far using diets containing extremely high levels of OTA, much higher than those in naturally contaminated feed materials (Gruber-Dorninger et al., 2019; Ishikuro, 2007; Ribelin et al., 1978; Shreeve et al., 1979).

Indeed, to assess the relevance of the effects of OTA in dairy cattle, the occurrence of this mycotoxin in animal feed must be considered. The data from a ten-year global mycotoxin survey showed that the occurrence of OTA in animal feeds and feedstuffs was 15 % (4858/32,271) with a maximum contamination level of 2000 μg/kg (Gruber-Dorninger et al., 2019). In a recent study, feed rations were sampled on 100 Austrian dairy farms and the presence of OTA was detected in only 1 % of the samples (at concentrations <8 μg/kg) (Penagos-Tabares et al., 2022). Ribelin et al. (1978) proposed that the lethal single oral dose of OTA for cattle is likely above 13 mg/kg of BW, a level unlikely to be encountered under practical conditions, even with highly contaminated grains. Consequently, acute OTA poisoning is considered rare in cattle farming.

In this study, the predominant excretion route of OTα was the faecal pathway, which underscores the bovine system's efficiency in managing OTA exposure through metabolic degradation to the less toxic OTα and elimination and the resilience of lactating Simmental cows to short-term exposure to high OTA levels in concentrate-rich diets. The low systemic OTA concentrations observed, together with the absence of OTA in milk and stable clinical parameters, likely reflect the efficient ruminal degradation and systemic detoxification capacity of lactating Simmental cows under short-term exposure conditions. These findings are consistent with previous studies reporting extensive ruminal biotransformation of OTA and limited systemic transfer in ruminants (Shreeve et al., 1979; Hashimoto et al., 2016 and Zhang et al., 2019, Mobashar et al., 2010, 2012) and evidenced in this study, with the high levels of OTAα in faeces. However, the observed variability among cows in metabolising and excreting OTA and OTα (Fig. S1) may be attributed to differences in ruminal microbiota composition and function. Although the current

data are insufficient to confirm this hypothesis, we speculate that cows with lower ruminal OTA-degrading capacity might be at greater risk of OTA toxicity. Hult et al. (1976) concluded that cows can tolerate OTA levels of up to 12 mg/kg in feed due to ruminal degradation, but this depends heavily on rumen functionality. The ability of cows to tolerate relatively high OTA levels without evident clinical effects is closely linked to the detoxification capacity of the rumen. The rumen microbiota can degrade OTA into its non-toxic metabolite OTα, thereby limiting its systemic absorption. This protective mechanism is well documented: premature ruminants, in which ruminal function is not fully established, exhibit markedly higher susceptibility to OTA toxicity, including clinical signs and, at higher doses, even mortality (Sreemannarayana et al., 1988). The efficiency of OTA degradation in the rumen is not fixed but influenced by dietary factors, particularly the concentrate-to-forage ratio and overall rumen environment, which can modulate microbial activity and detoxification capacity (Xiao et al. 1991 a,b; Müller et al. 2001; Fink-Gremmels, 2008; EFSA 2023). These findings emphasise the critical role of rumen function in OTA detoxification and the need to consider diet composition when assessing OTA risk in ruminants.

5. Conclusion

This study underscores that a short-term exposure to OTA, at doses of 5 mg and 50 mg per cow daily over seven days, had minimal effect on the performance and health parameters of Simmental dairy cows. Minor changes were observed in the group receiving the high OTA dose (equivalent to approx. 2500 mg/kg DM, assuming a daily feed intake of 20 kg DM) in faecal score, chewing activity and the composition of SCFAs in faeces. This finding of no adverse effect is likely linked to the natural degradation of OTA into OTα and L-β-phenylalanine in the rumen; the hypothesis that high levels of OTA exposure could lead to adverse health effects was not supported. It would be of interest to investigate the effect of OTA on different feeding regimes, which could impact the density and activity of the ruminal microbiota responsible for OTA degradation. This experiment involved acute exposure to OTA over seven days; however, acute intoxication rarely occurs, as high doses are rarely found in feed under practical conditions. Furthermore, as in reality, cows are exposed to multiple mycotoxins in their diets, it would be worth understanding about the interactions of other mycotoxins with OTA, as these could, for example, increase the adverse effects of OTA even at low doses. The toxicokinetic data presented here confirm that most OTA is metabolised to OTα, which is then in urine and faeces. These insights enhance our understanding of the resilience of ruminants to mycotoxin exposure and have implications for the formulation of livestock diets to ensure food safety and animal health. This study also demonstrated that estimating OTA and OTα in biological matrices is a suitable biomarker in ruminants, with plasma and serum as appropriate matrices that yield similar results. In addition, in lactating cows, OTα estimation may also serve as a reliable indicator of acute OTA exposure.

CRediT authorship contribution statement

Barbara Streit: Writing – review & editing, Software, Investigation, Formal analysis, Data curation. **Metzler-Zebeli Barbara U.:** Writing – review & editing, Supervision, Resources, Methodology. **Ratchaneewan Khiaosa-Ard:** Writing – review & editing, Software, Formal analysis, Data curation. **Siska Aditya:** Writing – review & editing, Investigation. **Emmanuela Gabara:** Writing – review & editing, Investigation. **Mubarik Mahmood:** Writing – review & editing, Methodology, Investigation. **Atif Rana-Muhammad:** Writing – review & editing, Resources, Investigation. **Johann Huber:** Writing – review & editing, Supervision, Investigation. **Raul Rivera-Chacon:** Writing – review & editing, Resources, Investigation. **Johannes Faas:** Writing – review & editing, Methodology, Conceptualization. **Qendrim Zebeli:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Felipe Penagos-Tabares:** Writing – original draft, Visualization, Validation, Project administration, Investigation, Formal analysis, Data curation. **Shreenath Prasad:** Writing – review & editing, Resources, Methodology, Conceptualization.

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Declaration of Competing Interest

I would like to declare that the authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The following authors B.S., J. F. and S.P. are employed by BIOMIN Holding GmbH (part of DSM), which operates the BIOMIN Research Center and is a manufacturer of feed additives. This, however, did not influence sampling, results presentation, or data interpretation.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2025.106541.

Data availability

All data supporting the findings of this study are available in the paper and its Supplementary Information section. Raw data are available from the corresponding author upon reasonable request.

References

Battacone, G., Nudda, A., Pulina, G., 2010. Effects of ochratoxin A on livestock production. Toxin 2, 1796–1824. https://doi.org/10.3390/toxins2071796.

Baumgartner, W., 2009. Klinische Propädeutik der Haus-und Heimtiere. Georg Thieme Verlag, Stuttgart.

Boudra, H., Barnouin, J., Dragacci, S., Morgavi, D., 2007. Aflatoxin M1 and ochratoxin A in raw bulk milk from French dairy herds. J. Dairy Sci. 90, 3197–3201. https://doi.org/10.3168/jds.2006-565.

Boudra, H., Saivin, S., Buffière, C., Morgavi, D., 2013. Toxicokinetics of ochratoxin A in dairy ewes and carryover to milk following a single or long-term ingestion of contaminated feed. J. Dairy Sci. 96, 6690–6696. https://doi.org/10.3168/jds.2013-6707.

Braun, U., Zürcher, S., Hässig, M., 2015. Evaluation of eating and rumination behaviour in 300 cows of three different breeds using a noseband pressure sensor. BMC Vet. Res. 11, 321. https://doi.org/10.1186/s12917-015-0549-8.

Douglas, J.W., Wardrop, K.J., 2010. Schalm's veterinary hematology. Wiley-Blackwell, Philadelphia.

European Food Safety Authority (EFSA) FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017. Guidance on the assessment of the safety of feed additives for the target species. EFSA J. 15, 5021. https://doi.org/10.2903/j.efsa.2017.5021.

European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM), Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., et al., 2023. Risks for animal health related to the presence of ochratoxin A (OTA) in feed. EFSA J. 21, e08375. https://doi.org/10.2903/j.efsa.2023.8375.

European Commission, 2006. Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (2006/576/EC). In: Off. J. Eur. Union, 229, pp. 7–9. ⟨https://eur-lex.europa.eu/legal-content/DE/TXT/PDF/?uri=CELEX:32006H0576⟩. Accessed 25 June 2025.

Food and Drugs Administration, 2015. Guidance for industry—studies to evaluate the metabolism and residue, kinetics of veterinary drugs in food-producing animals: validation of analytical methods used in residue depletion studies. U.S. Department of Health and Human Services, Washington. ⟨https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-208-vich-gl49-studies-evaluate-metabolism-and-residue-kinetics-veterinary-drugs-food⟩. Accessed 25 June 2025.

Fink-Gremmels, J., 2008. Mycotoxins in cattle feeds and carry-over to dairy milk: a review. Food Addit Contam Part A Chem. Anal. Control Expo. Risk Assess 25, 172. https://doi.org/10.1080/02652030701823142.

Firkins, J.L., Mitchell, K.E., White, A.F., 2024. Invited review: role for isoacids in dairy nutrition. AAS 40, 466–477. https://doi.org/10.15232/aas.2024-02537.

George, J.W., Snipes, J., Lane, V.M., 2010. Comparison of bovine hematology reference intervals from 1957 to 2006. Vet. Clin. Pathol. 39, 138–148. https://doi.org/10.1111/j.1939-165X.2009.00208.x.

Gruber-Dorninger, C., Jenkins, T., Schatzmayr, G., 2019. Global mycotoxin occurrence in feed: A ten-year survey. Toxins 11, 375. https://doi.org/10.3390/toxins11070375.

Gupta, R., 2007. Ochratoxins and citrinin. In: Gupta, R.C. (Ed.), Veterinary Toxicology: Basic and Clinical Principles. Elsevier: Academic Press, New York, London, pp. 997–1003.

Hartinger, T., Grabher, L., Pacífico, C., Angelmayr, B., Faas, J., Zebeli, Q., 2022. Short-term exposure to the mycotoxins zearalenone or fumonisins affects rumen fermentation and microbiota, and health variables in cattle. In: Food Chem. Toxicol, 162, 112900. https://doi.org/10.1016/j.fct.2022.112900.

Hashimoto, Y., Katsunuma, Y., Nunokawa, M., Minato, H., Yonemochi, C., 2016. Influence of repeated ochratoxin A ingestion on milk production and its carry-over into the milk, blood and tissues of lactating cows. Animal Sci. J. 87, 541–546. https://doi.org/10.1111/asj.12466.

Heussner, A.H., Bingle, L.E., 2015. Comparative ochratoxin toxicity: a review of the available data. Toxins 7, 4253–4282. https://doi.org/10.3390/toxins7104253.

Hulsen, J., 2005. Cow signals: a practical guide for dairy farm management. Roodbont Publishers.

Hult, K., Teiling, A., Gatenbeck, S., 1976. Degradation of ochratoxin A by a ruminant. Appl. Environ. Microbiol. 32, 443–444. https://doi.org/10.1128/aem.32.3.443-444.1976.

International Agency for Research on Cancer (IARC), 1993. Monographs on the evaluation of carcinogenic risks to humans: some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins, 56. IARC, Lyon, France, pp. 489–524.

Ishikuro, E., 2007. The current state of contamination and regulation of mycotoxins in feed in Japan. Mycotoxins 57, 123–127. https://doi.org/10.2520/myco.57.123.

Kiessling, K.H., Pettersson, H., Sandholm, K., Olsen, M., 1984. Metabolism of aflatoxin, ochratoxin, zearalenone, and three trichothecenes by intact rumen fluid, rumen protozoa, and rumen bacteria. Appl. Environ. Microbiol. 47 (5), 1070–1073.

Keyvan, E., Yurdakul, O., Kocasarı, F., Tutun, H., Demirtaş, A., Kahraman, H.A., Şen, E., 2018. Detection of ochratoxin A in bulk tank milk. Kocatepe. Vet. J. 11 (3), 255–259. https://doi.org/10.30607/kvj.424808.

Kononoff, P., Heinrichs, A., Buckmaster, D., 2003. Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements. J. Dairy Sci. 86 (5), 1858–1863. https://doi.org/10.3168/jds.S0022-0302(03)73773-4.

Kraft, W., Dürr, U.M., 2013. Klinische Labordiagnostik in der Tiermedizin. Schattauer Verlag.

Kröger, I., Humer, E., Neubauer, V., Kraft, N., Ertl, P., Zebeli, Q., 2016. Validation of a noseband sensor system for monitoring ruminating activity in cows under different feeding regimens. Livestock Sci. 193, 118–122.

Kumar, P., Mahato, D.K., Sharma, B., Borah, R., Haque, S., Mahmud, M.C., Shah, A.K., Rawal, D., Bora, H., Bui, S., 2020. Ochratoxins in food and feed: occurrence and its impact on human health and management strategies. Toxicon 187, 151–162. https://doi.org/10.1016/j.toxicon.2020.08.031.

Ledinek, M., Gruber, L., Steininger, F., Fuerst-Waltl, B., Zottl, K., Royer, M., Krimberger, K., Mayerhofer, M., Egger-Danner, C., 2019. Analysis of lactating cows in commercial Austrian dairy farms: interrelationships between different efficiency and production traits, body condition score and energy balance. Ital. J. Anim. Sci. 723–733.

Littell, R.C., Henry, P.R., Ammerman, C.B., 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76, 1216–1231. https://doi.org/10.2527/1998.7641216x.

Malik, T.A., Mohini, M., Mir, S., Ganaie, B., Singh, D., Varun, T., Howal, S., Thakur, S., 2018. Somatic cells in relation to udder health and milk quality-a review. J. Anim. Health Prod. 6, 18–26. https://doi.org/10.17582/journal.jahp/2018/6.1.18.26.

Mobashar, M., Hummel, J., Blank, R., Südekum, K.H., 2010. Ochratoxin A in ruminants–a review on its degradation by gut microbes and effects on animals. Toxins 2, 809–839. https://doi.org/10.3390/toxins2040809.

Mobashar, M., Blank, R., Hummel, J., Westphal, A., Tholen, E., Südekum, K.H., 2012. Ruminal ochratoxin A degradation—Contribution of the different microbial populations and influence of diet. Anim. Feed Sci. Technol. 171, 85–97.

Mostrom, M.S., Jacobsen, B.J., 2020. Ruminant mycotoxicosis: an update. Vet. Clin. North Am. Food Anim. Pract. 36, 745–774. https://doi.org/10.1016/j.cvfa.2020.08.011.

Müller, H.M., Lerch, C., Müller, K., Eggert, W., 1998. Kinetic profiles of ochratoxin A and ochratoxin α during in vitro incubation in buffered forestomach and abomasal contents from cows. Nat. Toxins. 6, 251–258 https://doi.org/10.1002/(sici)1522-7189(199811/12)6:6<251::aid-nt35>3.0.co;2-p.

Müller, H.M., Müller, K., Steingass, H., 2001. Effect of feeding regime on the metabolism of ochratoxin A during the *in vitro* incubation in buffered rumen fluid from cows. Archives Anim. Nutr. 54, 265–279. https://doi.org/10.1080/17450390109381984.

Naumann, C., Bassler, R., 2012. Handbuch Der Landwirtschaftlichen Versuchs-Und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III Die Chemische Untersuchung von Futtermitte.

Neubauer, V., Petri, R.M., Humer, E., Kröger, I., Reisinger, N., Baumgartner, W., Wagner, M., Zebeli, Q., 2020. Starch-rich diet induced rumen acidosis and hindgut dysbiosis in dairy cows of different lactations. Animals (Basel) 10 (10), 1727. https://doi.org/10.3390/ani10101727. PMID: 32977653; PMCID: PMC7598178.

Özpinar, H., Augonyte, G., Drochner, W., 1999. Inactivation of ochratoxin in ruminal fluid with variation of pH-value and fermentation parameters in an *in vitro* system. Environ. Toxicol. Pharmacol. 7, 1–9. https://doi.org/10.1016/s1382-6689(98)00041-6.

Pantoja, J., Hulland, C., Ruegg, P., 2009. Dynamics of somatic cell counts and intramammary infections across the dry period. Prev. Vet. Med. 90, 43–54. https://doi.org/10.1016/j.prevetmed.2009.03.012.

Pattono, D., Gallo, P.F., Civera, T., 2011. Detection and quantification of Ochratoxin A in milk produced in organic farms. Food Chem. 127, 374–377. https://doi.org/10.1016/j.foodchem.2010.12.051.

Penagos-Tabares, F., Khiaosa-ard, R., Nagl, V., Faas, J., Jenkins, T., Sulyok, M., Zebeli, Q., 2022. Cocktails of Mycotoxins, Phytoestrogens, and Other Secondary Metabolites in Diets of Dairy Cows in Austria: Inferences from Diet Composition and Geo-Climatic Factors. Toxins. 14, 493. https://doi.org/10.3390/toxins14070493.

Qumar, M., Khiaosa-Ard, R., Pourazad, P., Wetzels, S.U., Klevenhusen, F., Kandler, W., Aschenbach, J.R., Zebeli, Q., 2016. Evidence of in vivo absorption of lactate and modulation of short chain fatty acid absorption from the reticulorumen of non-lactating cattle fed high concentrate diets. PLoS One 11, e0164192. https://doi.org/10.1371/journal.pone.0164192.

Ribelin, W., Fukushima, K., Still, P., 1978. The toxicity of ochratoxin to ruminants. Can. J. Comp. Med. 42, 172.

Rivera-Chacon, R., Hartinger, T., Castillo-Lopez, E., Lang, C., Penagos-Tabares, F., Mühleder, R., Atif, R.M., Faas, J., Zebeli, Q., Ricci, S., 2024. Duration of Zearalenone Exposure Has Implications on Health Parameters of Lactating Cows. Toxins 16, 116. https://doi.org/10.3390/toxins16030116.

Shevhuzhev, A., Belik, N., Emelyanov, E., Tokar, A., 2017. Milk productivity of Simmental cows Austrian selection. Eng. Rural. Dev. 24, 1354–1358. https://doi.org/10.22616/ERDev2017.16.N304.

Shreeve, B., Patterson, D., Roberts, B., 1979. The 'carry-over' of aflatoxin, ochratoxin and zearalenone from naturally contaminated feed to tissues, urine' of aflatoxin, ochratoxin, and zearalenone is from naturally contaminated feed to tissues, urine, and milk of dairy cows. Food Cosmet. Toxicol. 17, 151–152. https://doi.org/10.1016/0015-6264(79)90215-3.

Sreemannarayana, O., Frohlich, A., Vitti, T., Marquardt, R., Abramson, D., 1988. Studies of the tolerance and disposition of ochratoxin A in young calves. J. Anim. Sci. 66 (7), 1703–1711. https://doi.org/10.2527/jas1988.6671703x.

Streit, B., Czabany, T., Weingart, G., Marchetti-Deschmann, M., Prasad, S., 2022. Toolbox for the extraction and quantification of ochratoxin A and ochratoxin alpha applicable for different pig and poultry matrices. Toxins. 14, 432. https://doi.org/10.3390/toxins14070432.

Vettorazzi, A., González-Peñas, E., de Cerain, A.L., 2014. Ochratoxin A kinetics: A review of analytical methods and studies in rat model. Food Chem. Toxicol. 72, 273–288. https://doi.org/10.1016/j.fct.2014.07.020.

Xiao, H., Marquardt, R., Frohlich, A., Phillips, G., Vitti, T., 1991a. Effect of a hay and a grain diet on the bioavailability of ochratoxin A in the rumen of sheep. J. Anim. Sci. 69, 3715–3723. https://doi.org/10.2527/1991.6993715x.

Xiao, H., Marquardt, R., Frohlich, A., Phillips, G., Vitti, T., 1991b. Effect of a hay and a grain diet on the rate of hydrolysis of ochratoxin A in the rumen of sheep. J. Anim. Sci. 69, 3706–3714. https://doi.org/10.2527/1991.6993706x.

Zebeli, Q., Tafaj, M., Junck, B., Mansmann, D., Steingass, H., Drochner, W., 2008. Evaluation of the effects of dietary particle fractions on fermentation profile and concentration of microbiota in the rumen of dairy cows fed grass silage-based diets. Archives Anim. Nutr. 62, 230–240. https://doi.org/10.1080/17450390802027486.

Zhang, Z., Fan, Z., Nie, D., Zhao, Z., Han, Z., 2019. Analysis of the carry-over of ochratoxin A from feed to milk, blood, urine, and different tissues of dairy cows based on the establishment of a reliable LC-MS/MS method. Molecules 24, 2823. https://doi.org/10.3390/molecules24152823.

Zhang, Z., Song, Y., Ma, L., Huang, K., Liang, Z., 2022. Co-Occurrence of *Staphylococcus aureus* and Ochratoxin a pasteurized milk. Toxins. 14, 718. https://doi.org/10.3390/toxins14100718.