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Ruminal ochratoxin A degradation—Contribution of the different microbial populations and influence of diet

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ABSTRACT

The mycotoxin ochratoxin A (OTA) is degraded extensively in the rumen. In this study, the relative contribution of different rumen microbial populations (MP) and the effect of diet on degradation of OTA were evaluated in a factorial design experiment. Degradation of OTA was quantified by using the Hohenheim gas test (HGT) *in vitro* fermentation system. Five different HGT diets were used (concentrate:forage proportions (C:F) – 10:90, 30:70, 50:50, 70:30, 90:10), and donor animals were fed diets with the respective ratio. Diets with the highest concentrate content were supplied with and without 10 g/kg sodium bicarbonate (70:30 BC and 90:10 BC). The MP included whole rumen fluid, fungi + protozoa, bacteria + protozoa, protozoa and bacteria + fungi. Protozoa numbers were counted after 24 h and OTA and ochratoxin alpha (OTα) analysed at 0, 4, 8, 12, 24 h. Area under the curve (AUC) and half-life were calculated for the latter two. The short average OTA half-life for whole rumen fluid of 2.6 h (1.3–4.5 h) demonstrates the high OTA degradation capacity of the rumen MP (*i.e.*, standard HGT inoculum) and corresponds well with published *in vivo* results. Both MP and diet affected OTA degradation. Interactions among factors occurred ($P<0.001$), which made it necessary to do further comparisons within factor levels. Among MP, those with bacteria (bacteria + fungi and bacteria + protozoa) had lower AUC values ($P<0.001$) for OTA (196–673 ng/ml h, meaning higher degradation capacity, than those without bacteria (fungi + protozoa and protozoa; 701–1206 ng/ml h). Whole rumen fluid had the lowest AUC values (146–249 ng/ml h; $P<0.05$). Diet had a quadratic effect ($P=0.001$) on protozoal numbers with minimum values for the lowest and highest C:F ratios, for bacteria + protozoa, fungi + protozoa and protozoa, but no corresponding effect was found for OTA degradation parameters. While the generally high capacity to degrade OTA was confirmed, results for the contribution of different microbial groups shed new light on ruminal OTA degradation.

1. Introduction

Mycotoxins are a large diverse group of naturally occurring secondary metabolites of fungi. Among ochratoxins, ochratoxin A (OTA) is the most prevalent metabolite of some toxigenic species of *Aspergillus* and *Penicillium* genera (Chu,

Abbreviations: ADFom, acid detergent fibre expressed exclusive of residual ash; AUC, area under the curve; BC, sodium bicarbonate ($NaHCO_3$); B + F, bacteria + fungi; B + P, bacteria + protozoa; C:F, concentrate:forage; CP, crude protein; DM, dry matter; EE, ether extract; F + P, fungi + protozoa; HGT, Hohenheim gas test; HPLC, high performance liquid chromatography; MP, microbial population; aNDFom, neutral detergent fibre assayed with a heat-stable amylase and expressed exclusive of residual ash; OTA, ochratoxin A; OTα, OT alpha; P, protozoa; WRF, whole rumen fluid.

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1974; Frisvad and Samson, 1991). *Penicillium* tends to be more prevalent in cooler climates whereas in warmer climates *Aspergillus* is of more relevant (Krogh, 1987). Ochratoxin A has been found as a natural contaminant in food and feedstuffs (Shotwell et al., 1969; Dwivedi and Burns, 1986). Contamination levels of feedstuffs can be higher than 100 μg/kg (Kuiper-Goodmann and Scott, 1989), but most feeds which test positive will be below this level. While concentrates can be contaminated with OTA, forages are generally not considered as an important source of OTA (Mobashar et al., 2010). Therefore, the potential for OTA contamination of ruminant diets increases with the concentrate proportion of the diet.

Ochratoxin A is a complex compound containing ochratoxin α (OTα) which is linked via a 7-carboxy group to L-β-phenylalanine by an amide bond. Exposure to OTA causes a variety of pathological responses such as nephrotoxic, hepatotoxic, teratogenic and carcinogenic in non-ruminants, with nephropathy commonly occurring in swine (Krogh, 1987; Marquardt and Frohlich, 1992). However, ruminants are relatively resistant to its toxic effects due to detoxification of OTA to its less toxic metabolite, OTα and phenylalanine by rumen microbes (Ribelin et al., 1978; Kuiper-Goodmann and Scott, 1989; Blank et al., 2004; Driehuis et al., 2010; see Mobashar et al., 2010 for a recent review on OTA in ruminants). Several *in vitro* studies using ruminal fluid collected from cows and sheep, and administration of OTA into the rumen of calves, sheep and goats, have reported OTA hydrolytic capacity of rumen microbes (Hult et al., 1976; Nip and Chu, 1979; Müller et al., 1998). Based on *in vitro* estimations, ruminants are able to degrade up to 12 mg of OTA/kg contaminated feed (Hult et al., 1976; Kiessling et al., 1984). While such *in vitro* studies mostly do not consider passage of digesta out of rumen and the following absorption in the lower gastrointestinal tract (rumen escape), they have considerable merit in evaluating principles of ruminal degradation.

Former studies have indicated that 0.88–0.90 of total hydrolysis of OTA to less toxic metabolites is associated with rumen protozoa and only 0.10–0.12 with bacteria (Galtier and Alvinerie, 1976; Kiessling et al., 1984; Xiao et al., 1991). According to previous studies degradation and systemic availability of OTA depends on concentrate:forage (C:F) ratio in the diet, which in turn seems to be important to maintain composition of an active rumen microbial population (MP) with respect to OTA degradation.

This study aimed to evaluate effects of different diet types (C:F) and the contribution of different microbial groups (*i.e.*, bacteria, protozoa and fungi) in the rumen to OTA degradation. Given the general emphasis on the role of protozoa regarding OTA degradation, their role was of particular interest.

2. Materials and methods

2.1. Donor animals and feeding regime

Rumen fluid was obtained from three fistulated Blackface ewes (60 ± 10.0 kg body weight). Diets fed to the animals were (C:F ratio, dry matter (DM) basis) 10:90, 30:70, 50:50, 70:30 BC (sodium bicarbonate), 70:30, 90:10 BC and 90:10. Bicarbonate was added at 10 g/kg of the diet. The forage was chopped grass hay and the concentrate (g/kg) was 261 solvent-extracted rapeseed meal, 180 palm kernel expeller, 180 wheat middlings, 150 wheat gluten feed, 141 soybean hulls, 70 beet vinasse, 16 $CaCO_3$ and 2 NaCl. The diet was given in two meals at 07:00 h and 15:30 h. Ewes were fed each diet for a 10–14 days adaptation period. Water was available *ad libitum*. The chemical composition of donor animal diets calculated from the means of concentrate and hay samples of all periods is in Table 1. The average chemical composition (±SD; g/kg DM) of concentrate over all periods was: ash 86 ± 3.4, crude protein (CP) 240 ± 8.1, ether extract (EE) 42 ± 6.7, neutral detergent fibre (assayed with a heat-stable amylase and expressed exclusive of residual ash; aNDFom) 375 ± 3.4, acid detergent fibre (expressed exclusive of residual ash, ADFom) 192 ± 26.2 and lignin(sa) 69 ± 12.4, and for hay: ash 105 ± 5.9, CP 123 ± 8.6, EE 27 ± 6.2, aNDFom 602 ± 21.6, ADFom 307 ± 28.0 and lignin(sa) 59 ± 6.7.

Table 1
Dry matter (DM) content (g/kg) and composition[a] (g/kg DM) of donor animal diets.

Diet (C:F[b])	DM (g/kg)	Ash (g/kg DM)	CP (g/kg DM)	EE (g/kg DM)	aNDFom (g/kg DM)	ADFom (g/kg DM)	Lignin(sa) (g/kg DM)	NFC[c] (g/kg DM)
10:90	909	103	135	28.3	579	296	65.4	155
30:70	906	99.6	158	31.5	534	273	61.9	177
50:50	903	95.8	181	34.6	488	250	63.9	201
70:30 BC[d]	890	91.1	203	37.3	438	224	65.3	231
70:30	899	92.1	205	37.7	443	227	66.0	222
90:10 BC	887	87.4	226	40.4	393	202	67.3	253
90:10	896	88.3	228	40.8	397	204	68.0	246

[a] CP, crude protein; EE, ether extract; aNDFom, neutral-detergent fibre assayed with a heat-stable amylase and expressed exclusive residual ash; ADFom, acid-detergent fibre expressed exclusive residual ash; NFC, non-fibre carbohydrate (1000 − aNDFom − CP − EE − Ash).
[b] Concentrate:forage.
[c] NFC = non-fibre carbohydrate (1000 − aNDFom − CP − EE − ash).
[d] Sodium bicarbonate.