

## Effect of roughage:concentrate ratio in the diet on systemic availability and excretion of ochratoxin A in sheep\*

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### ABSTRACT

Microbial detoxification of ochratoxin A in the rumen may be influenced by the concentrate level in the diet. The aim of the present study was to evaluate the effects of practically relevant concentrate levels in ruminant diets on the metabolism of ochratoxin A in sheep. High concentrate diets delayed ruminal degradation of ochratoxin A to the less toxic ochratoxin  $\alpha$  and increased the systemic availability of ochratoxin A compared to high roughage diets, implying that under intensive ruminant production conditions the potential for a carry over of ochratoxin A into meat or milk may be increased.

KEY WORDS: ochratoxin A, ochratoxin  $\alpha$ , mycotoxin, sheep, ruminants, metabolism, excretion

### INTRODUCTION

Ruminants are relatively resistant to acutely toxic effects of the mycotoxin ochratoxin A (OA), due to extensive ruminal degradation of OA to its less toxic metabolite ochratoxin  $\alpha$  (O $\alpha$ ). The observation that calves with an intact rumen are more tolerant to orally administered OA as compared to preruminant calves yields further evidence that ruminal hydrolysis of OA to O $\alpha$  might be an important detoxification process in ruminants (Sreemannarayana et al., 1988). These findings have led to the hypothesis that feeding OA-contaminated feedstuffs to

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ruminants may be an efficient means for using these feedstuffs and to minimize the toxic effects and the carry over of OA in the food chain. From *in vitro* studies it has been estimated that the capacity of dairy cows to degrade OA is in the range of 33-72 mg/d and that sheep should be able to degrade 3-7 mg/d (Müller, 1995). However, recent *in vivo* studies with sheep indicated that ruminal hydrolysis of OA to O $\alpha$  might be lower than estimated from *in vitro* studies. Even at a low OA intake of ~0.4 mg, relevant concentrations of intact OA were found in blood serum (Blank et al., 2003). Furthermore, a complete concentrate diet resulted in a 4.3-fold increase of the systemic availability of OA compared to a complete hay diet (Xiao et al., 1991). The aim of the present study was to investigate the effect of diets with practically relevant concentrate:roughage ratios on the systemic availability, ruminal degradation, and excretion of OA after chronic feeding of naturally occurring concentrations of OA.

## MATERIAL AND METHODS

Twelve castrated male sheep with an average body weight (BW) of 58.0 $\pm$ 6.0 kg were used in this experiment. The animals were housed individually in metabolism crates in a temperature-controlled room. Water was freely available from drinking bowls. The animals were divided into four groups of 3 sheep each. Dietary treatments consisted of a high concentrate diet (70%) without or with OA (14  $\mu$ g/kg BW) and high roughage diet (70%) without or with OA (14  $\mu$ g/kg BW). The animals were fed twice daily at 8.00 and 19.00. The energy and protein supply was 1.4 times the requirements for maintenance (GfE, 1996). The experiment was carried out according to a two-period changeover design. Each experimental period lasted 31 d. The following samples were taken in each experimental period, respectively. Blood samples from the jugular vein were taken on d 1, 5, 9, 13, 23, and 29 prior to the morning feeding. Faeces and urine were quantitatively collected for a period of 7 d from d 15 to 22. Additionally, ruminal fluid was collected on d 24 to 25 at various time points after feeding using an oro-ruminal probe. Methods for extraction and analysis of OA from blood serum, ruminal fluid, faeces and urine by HPLC using fluorescence detection have been previously described (Blank et al., 2003).

## RESULTS AND DISCUSSION

None of the animals developed any overt illness or health disturbance related to OA treatment. The daily amount of feed offered was always completely consumed within 1 h during the entire experiment. Ochratoxin A was not detected in blood serum, ruminal fluid, faeces, and urine of the animals receiving the control diets without OA, indicating that the feedstuffs were not

contaminated. The high concentrate diets decreased ( $P<0.05$ ) the post-feeding ruminal pH compared to the roughage diets (data not shown). This effect was associated with a delayed disappearance of OA from the rumen (Figure 1a) and an increase of OA concentration in blood serum (Figure 1b). The total excretion of OA and O $\alpha$  in urine and faeces was not affected by the concentrate level in the diet (Table 1). However, compared to the roughage diet, the fractional renal excretion of O $\alpha$  increased in animals receiving the high concentrate diet. This effect may be due to higher biliary excretion of absorbed OA and subsequent microbial hydrolysis of OA to O $\alpha$  in the large intestine. Nutrient digestibility increased with increasing proportion of concentrate in the diet and were not affected by OA (data not shown).

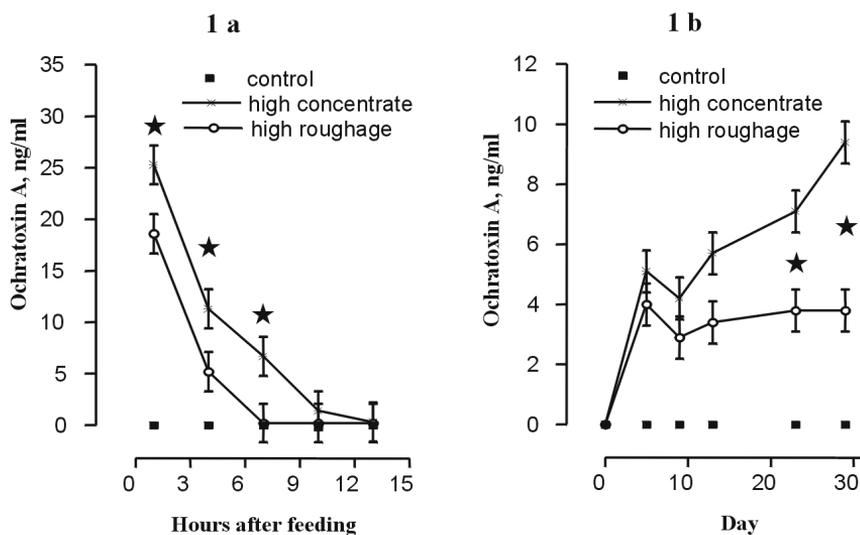


Figure 1. a. Concentrations of ochratoxin A in ruminal fluid at various time points after feeding. b. Concentration of ochratoxin A in blood serum (★ denotes significant ( $P<0.05$ ) difference between high concentrate and high roughage group)

Our results are in agreement with previous studies investigating the effect of high concentrate diets on ruminal degradation of OA (Xiao et al., 1991). According to Kiessling et al. (1984) ruminal hydrolysis of OA is mainly attributed to the protozoa in the rumen. High concentrate diets decrease ruminal pH which may reduce the activity of protozoa and thereby decreasing the hydrolysis of OA. In addition, OA is a weak acid which is mainly absorbed passively in the nondissociated form (Kumagai, 1988). Thus, the lowered ruminal pH might have favoured absorption of OA from the rumen.

Table 1. Proportional excretion of OA and O $\alpha$  in faeces and urine, % applied amount

	Roughage/concentrate without OA	Roughage 14.3 $\mu$ g OA/kg BW	Concentrate 14.3 $\mu$ g OA/ kg BW	S. E.M.
Faeces				
OA	-	1.2	0.7	0.1
O $\alpha$	-	9.1 <sup>a</sup>	14.4 <sup>b</sup>	0.4
total	-	10.3 <sup>a</sup>	15.1 <sup>b</sup>	0.5
Urine				
OA	-	3.6	4.6	0.6
O $\alpha$	-	67.5 <sup>a</sup>	58.2 <sup>b</sup>	1.5
total	-	71.1 <sup>a</sup>	62.9 <sup>b</sup>	1.5
Faeces + urine	-	81.4	78.0	1.5

<sup>a,b</sup> least squares means in the same row with different superscripts differ significantly (P<0.05)

## CONCLUSIONS

The results of this study indicate that high concentrate diets delayed ruminal degradation of OA and increased its systemic availability compared to high roughage diets. This implies that under intensive ruminant production conditions the potential for a carry over of OA into meat or milk may be increased.

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