Research Article

Effects of the level of feed intake and ergot contaminated concentrate on ergot alkaloid metabolism and carry over into milk

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The aim of the present study was to examine the effects of ergot contaminated concentrate at differing levels of feed intake on ergot alkaloid metabolism and carry over into milk. Twelve double fistulated (in the rumen and the proximal duodenum) Holstein Friesian cows were fed either the control diet (on a dry matter (DM) base: 60% maize silage, 40% concentrate) or the contaminated diet (concentrate contained 2.25% ergot, which caused an alkaloid concentration of the daily ration between 504.9 and 619.5 μg/kg DM) over a period of 4 weeks. Daily feed amounts were adjusted to the current performance which resulted in a dry matter intake (DMI) variation between 6.0 and 18.5 kg/day. The actual alkaloid exposure varied between 4.1 and 16.3 μg/kg body weight when the ergot contaminated concentrate was fed. Approximately 67% of the alkaloids fed were recovered in the duodenal ingesta, and ~24% were excreted with the faeces. No alkaloid residues could be detected in the blood or milk samples.

Keywords: Carry over / Dairy cow / Ergot alkaloid metabolism / Feed intake / Milk

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1 Introduction

The solidified mycelium of *Claviceps purpurea* is called ergot and its presence in feed might affect performance and can even lead to intoxication symptoms in ruminants [1].

Full-grown ruminants are considered as less susceptible to mycotoxins due to the detoxifying potential of their rumen microbes. These microbes again may be influenced in their activity by varying feed intake and passage rates through the rumen [2]. The level of feed or organic matter intake of a cow normally shows a high variation over the year, dependent on the stage of lactation. With an increasing intake of organic matter, the retention time in the rumen decreases and the passage rate in turn increases, which might influence the time available for the metabolisation of mycotoxins such as ergot alkaloids. Moreover, the pH, and probably the proteolytic and cellulolytic activities in the rumen, are thought to be influenced by the level of feed intake, which might in turn could influence the alkaloid metabolism and/or absorption [3, 4].

The present study was conducted to examine the effects of an ergot contaminated concentrate on alkaloid concentrations and profiles at the proximal duodenum at differing levels of feed intake and to investigate the ruminal ergot alkaloid metabolism, as well as to assess the potential absorption of these alkaloids. Furthermore, blood and milk samples were collected to prove whether alkaloids reach the systemic circulation and the milk.

2 Material and methods

2.1 Experimental design and animals

The study was conducted with twelve dairy cows of the "Holstein Friesian" breed fitted with large rubber cannulas in the dorsal sac of the rumen (id: 10 cm) and simple T-shaped plastic cannulas at the proximal duodenum close to the pylorus (id: 2 cm). The cows had a mean body weight of 610 (±82) kg at the beginning of the study and were housed in a tethered-stall with neck straps. Each cow box was equipped with an individual trough and had free access to water and a salt block containing sodium chloride. The diets consisted of 40% concentrate and 60% maize silage on a

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Abbreviations: DM, dry matter; **DMI,** dry matter intake

Table 1. Composition of the concentrates (g/kg as fed)

Components	Group	
	Control	Ergot
Soybean meal	200	200
Barley	100	100
Wheat	260	255
Dried sugar beet pulp	230	230
Peas	150	150
Calcium carbonate	18	18
Soybean oil	10	10
Urea	12	12
Mineral and vitamin premix[a)]	20	20
Ergoty rye[b)]	0	5

a) Provided *per* kg concentrate: 175 g Ca, 100 g Na, 50 g P, 30 g Mg, 1.5 g Fe, 2 g Mn, 6 g Zn, 1.2 g Cu, 30 mg I, 20 mg Co, 40 mg Se, 1 000 000 IU vitamin A, 100 000 IU vitamin D_3, 2000 IU vitamin E.
b) 45% Ergot.

dry matter (DM) basis. All animals were fed the same diet type, whereas the daily amount was adjusted according to the current performance and production stage in order to cover a wide range in dry matter intake (DMI). Maize silage was given in two equal portions at 5:00 and 15:00 h. The concentrate was evenly distributed over four feeding times *per* day at 5:00, 8:00, 15:00 and 17:00 h. The composition of the concentrates is shown in Table 1. Lactating cows ($n = 8$) were milked at 5:00 and 16:00 h.

The experiment was split into two treatment periods where either the control or the ergot containing concentrate (Table 1) was fed. Each cow passed through both treatments excepting one cow which was replaced by another cow due to health problems.

2.2 Sample collection and measurements

Each treatment period consisted of 4 weeks. Two weeks of adaptation to the diets were followed by duodenal chyme collection. Over a period of 5 days chyme samples were taken at 2 h intervals through the duodenal cannula. Cr_2O_3 was used as a marker, given every 12 h. into the rumen beginning 10 days before the duodenal sampling period, and every 6 h. during the sampling period to estimate the duodenal flow. Immediately after the collection, pH-values were measured with a glass electrode (digital pH measurement device, pH525, WTW) in the four 100 mL-samples taken *per* cow. The one with the lowest pH was added to the daily pooled sample of each cow as described by Rohr *et al.* [5].

Blood samples were collected once *per* treatment period during week four at 10:00 h to cover the peak of the serum alkaloid curve, which occurs ~2 h after the start of feeding [6]. The tubes were centrifuged at $2000 \times g$ and 15°C for 10 min ~2 h after sampling and afterwards stored in a deep freezer. The cows were weighed on the day before the experiment started and on the last day. Milk yield was recorded at each milking time and samples for ergot alkaloid analyses were taken through four consecutive morning and evening milkings at week four of each treatment period.

Urine (spontaneous micturition) and faeces (rectally collected) of each cow were sampled once in week four.

Samples of maize silage, concentrates and feed refusals, if occurring, were collected daily during the ingesta sampling weeks, pooled and dried at 60°C. Samples of the faeces, the milk and the duodenal ingesta were freeze-dried and, just as the dried samples of the feedstuffs, ground to 1 mm for analysis.

2.3 Analysis

The chemical composition of the feedstuffs was analysed according to the methods of the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA) [7]. Ergot and feedstuffs were analysed for ergot alkaloids (ergometrine, ergocornine, ergotamine, α-ergocryptine, ergosine, ergocristine, and their –inine isomers) by HPLC based on the method by Wolff *et al.* [8].

Approximately 3 g of the samples were mixed with 100 mL extraction fluid (50 mL dichlormethane + 25 mL ethylacetate + 5 mL methanol + 1 mL ammonium hydroxide (25%)) and the day after centrifugation an aliquot was taken and evaporated to dryness. The next step was to dissolve the residue in 2 mL toluene/methanol (49 + 1) and to solubilise by ultrasound. The fluid was mixed with 9 mL i-hexane and added to a 3 g Extrelut® column (Merck, Darmstadt, Germany), which was acidified with 5 mL 2% aqueous tartaric acid. For the following elution, 0.5 mL toluol/methanol + 4.5 mL i-hexane and 20 mL di-isopropylether/i-hexane (1 + 1) were used. For 1–2 min air was sucked through to dry the column before the alkaloids were assimilated in 25% ammonium gas, which was detected by colour reaction of phenolphthalein. The alkaloids were eluted with 25 mL dichlormethane, which was evaporated to dryness at 35°C and carefully blown off with nitrogen. Finally the residue was filled up to a definite volume of 20 µL and was injected in the HPLC-apparatus. The HPLC consists of an isocratic pumping system with a 250 mm × 4 mm column (5 µm, C 18 Gravity, Macherey-Nagel, Düren, Germany), operates at 44°C and is connected with a fluorescence detector (325 nm excitation/418 nm emission wavelength).

The serum samples, milk, urine and faeces were analysed with the same method.

The LOD was 5 ng/g, except for ergometrine where it was 10 ng/g. Ergometrine, ergotamine, ergocristine, ergocornine, and ergocryptine are referred as to "key alkaloids", since standards were commercially available for their identification. Ergosine and its isomer were identified by their retention time [9].

Contaminations with zearalenone (ZON) were analysed according to a modified VDLUFA method according to Ueberschär [10] as described by Dänicke *et al.* [11].

The Cr_2O_3 in marker and ingesta samples was determined using atomic absorption spectrophotometry as described by Williams [12], and was used to estimate the daily ingesta flow and thus the daily flow of ergot alkaloids.

2.4 Calculations and statistics

For the calculation of the DM flow (DMF) the following formula was used:

$$\mathrm{DMF(kg/day)} = \frac{\text{chromium application(mg/day)}}{\text{duodenal chromium concentration(mg/gDM)}} / 1000$$

The daily duodenal flows of ergot alkaloids were estimated by the multiplication of their chyme concentrations with the DMF.

ME in the feedstuffs was calculated as described by the GfE [13] using the prediction equation

$$\mathrm{ME(MJ)} = 0.0312 \times \mathrm{gDXL} + 0.0136 \times \mathrm{gDXF} + 0.0147 \times \mathrm{g(DOM} - \mathrm{DXL} - \mathrm{DXF}) + 0.00234 \times \mathrm{gXP}$$

The daily amount of faecal organic matter was determined by estimating the amount of organic matter intake and the digestibilities given by the DLG [14]. The daily alkaloid excretion with the faeces could thus be calculated by multiplying the estimated amount of faecal organic matter by the alkaloid concentration in the organic matter of the sample.

As treatment comparisons were not applicable due to the fact that no alkaloid residues were detectable in the investigated physiological specimens collected from the cows during the control period the statistics were confined to linear regressions to describe the relationships between alkaloid intake and alkaloid flow or excretion. Regressions were performed by using the Statistica for the Windows™ operating system [15].

Treatments and experiment were conducted according to the European Community regulations concerning the protection of experimental animals and were approved by the Land Bureau for Consumer Protection and Food Safety for Lower Saxony (LAVES) in Oldenburg, Germany (File Number 509.42502/09-02.02).

3 Results

3.1 Chemical composition of the feedstuffs

The nutrient composition of the feedstuffs pooled over the course of the study was almost comparable (Table 2).

The ergot used in the present experiment had a mean alkaloid concentration of 681 mg/kg DM with ergotamine, ergocristine and ergosine together amounting to more than 50% (data not shown).

The total alkaloid content of the ergotised concentrate varied between 930 and 1484 μg/kg DM with ergotamine, ergocristine, ergosine and its isomers being the most prominent alkaloids (Table 2). The alkaloid content of the maize silage over the entire experimental period was lower than the indicated LODs, whereas the concentrate of the Control treatment showed a mean alkaloid content of 7 μg/kg DM (0 – 53 μg/kg DM).

Table 2. Mean values ($n = 6$) of DM (g/kg), nutrient composition (g/kg DM), energy concentration (MJ ME/kg DM) and alkaloid pattern of the concentrates (μg/kg DM; percentage of total alkaloids in brackets)

	Concentrate		Maize silage
	Control	Ergot	
Nutrients and energy (g/kg DM)			
DM (g/kg)	878.0	882.4	966.7
Crude protein	222.6	229.9	77.1
Crude fat	28.2	29.3	29.8
Crude fibre	76.5	81.2	181.5
ME [MJ/kg]	12.8	12.9	10.7
Alkaloids (μg/kg DM (%))			
E-metrine	2.2 (29)	88 (6)	<10
E-metrinine	0	18 (1)	<10
E-amine	1.7 (22)	347 (24)	<5
E-aminine	0.8 (11)	191 (13)	<5
E-cornine	0	72 (5)	<5
E-corninine	0	39 (3)	<5
E-cryptine	0	67 (5)	<5
E-cryptinine	0	60 (4)	<5
E-cristine	1.2 (16)	231 (16)	<5
E-cristinine	0.4 (5)	103 (7)	<5
E-sine	0.9 (12)	143 (10)	<5
E-sinine	0.4 (5)	70 (5)	<5
Total alkaloids	7.6	1430	<70

In all of the feedstuffs, the content of β-zearalenol was lower than the LOD of 5 ng/g DM. In pooled samples of the maize silage 2.3 ng α-zearalenol/g and 139.4 ng zearalenone/g on a DM basis were detected. The concentrates showed a-zearalenol contents which were lower than the LOD of 1 ng/g DM. Zearalenone was detected with 5.6 ng/g DM in the Control concentrate and with 4.6 ng/g DM in the ergotised concentrate.

On a DM basis, deoxynivalenol was detected in the maize silage with a concentration of 1858 ng/g. The deoxynivalenol contents of the concentrates were lower than the LOD of 34 ng/g DM.

3.2 Alkaloid metabolism and excretion

No alkaloid residues were detectable during the control period where the cows were fed the concentrate without ergot in any of the analysed physiological specimens. The alkaloid concentration of the ergot supplemented concentrate ranged between 504.9 and 619.5 μg/kg DM of the daily ration and corresponded to an exposure between 4.1 and 16.3 μg/kg live weight (BW). The mean recovery rates in the duodenal samples ranged between 78 and 111%, and in the faeces between 63 and 108%.

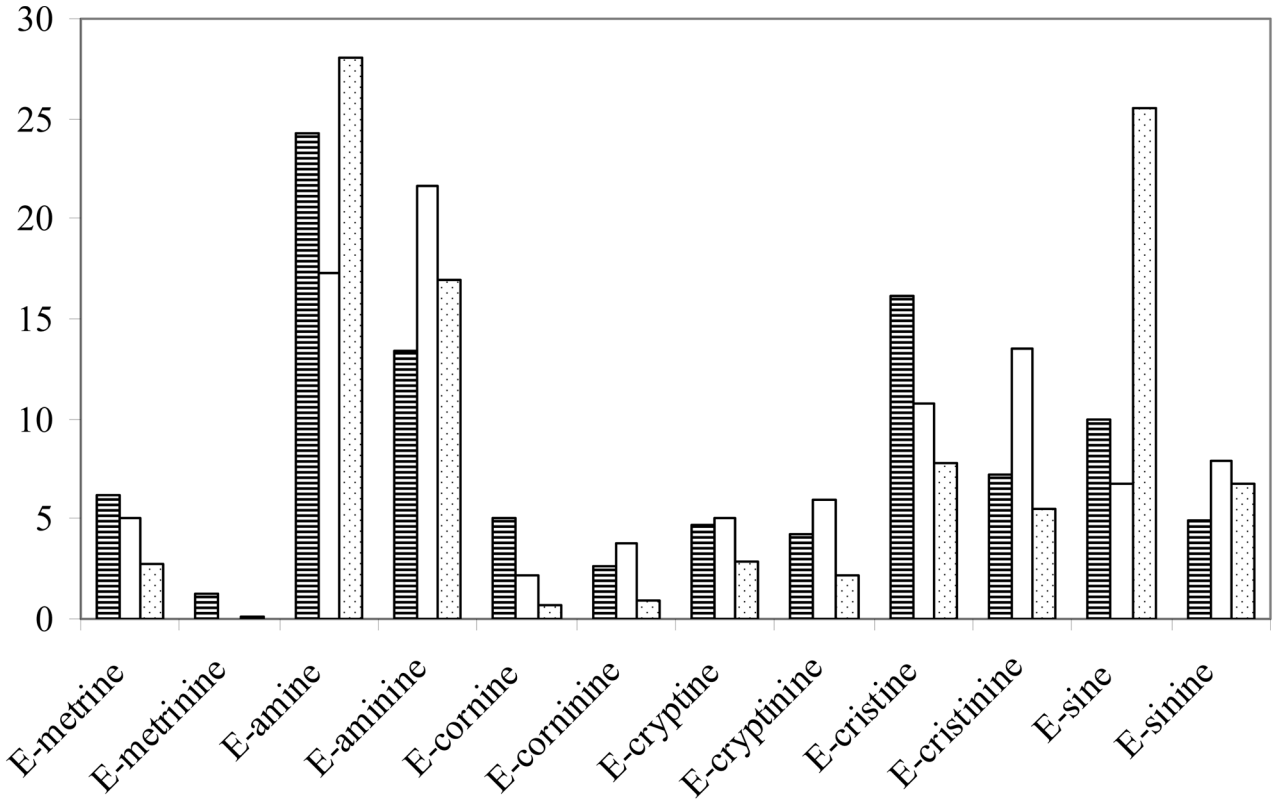

Figure 1. Mean alkaloid proportions (% of total alkaloids) in feedstuff (striped), duodenal chyme (white) and faeces (spotted) of dairy cows fed an ergot supplemented concentrate.

Table 3. Alkaloid exposure, alkaloid flow at the duodenum and alkaloid excretion via faeces of cows fed the ergoty concentrate

Alkaloid exposure		Alkaloid flow to the duodenum		Alkaloid flow to the faeces	
(μg/day)	(μg/kg BW)	(μg/day)	(% of intake)	(μg/day)	(% of intake)
3583.3	4.1	2594.1	72.4	600.9	16.8
4200.0	5.5	2288.5	54.5	904.7	21.5
5593.2	7.8	4642.3	83.0	1899.7	34.0
5888.9	10.2	2848.0	48.4	1252.4	21.3
6481.5	10.6	4988.9	77.0	1305.6	20.1
8576.4	13.9	5911.3	68.9	2066.5	24.1
7695.0	14.0	5445.7	70.8	1458.3	19.0
8268.0	14.2	3722.3	45.0	1975.9	23.9
8325.8	14.6	7271.9	87.3	1543.4	18.5
8760.0	15.2	5940.0	67.8	3350.4	38.2
7468.4	15.3	4381.5	58.7	966.2	12.9
10213.1	16.3	7052.5	69.1	3290.3	32.2

The mean total amount of alkaloid intake amounted to 7088 ± 1974 μg/day. Approximately 67 ± 13% of it were recovered in the duodenal ingesta and ~24 ± 8% were excreted with the faeces. The mean percentage of –in isomers in total alkaloids was 73 ± 7% in the concentrate, 48 ± 3% in the duodenal chyme and 68 ± 8% in the faeces (Table 3).

The mean alkaloid proportions in feedstuff, duodenal chyme and faeces, and the relationship between alkaloid content of the duodenal chyme and the faeces, are shown in Figs. 1 – 3.

Urine alkaloid contents were lower than the detection rates of the method used.

3.3 Ergot residues and carry over

Mean recovery rates in the milk samples ranged between 62 and 86%, and in the blood between 77 and 106%. Neither the analysed blood samples, nor the pooled milk samples of the lactating cows contained detectable amounts of alkaloids.

4 Discussion

4.1 Chemical composition of the feedstuffs

The nutrient composition and energy content of the concentrates, as shown on Table 2, were almost equal and thus

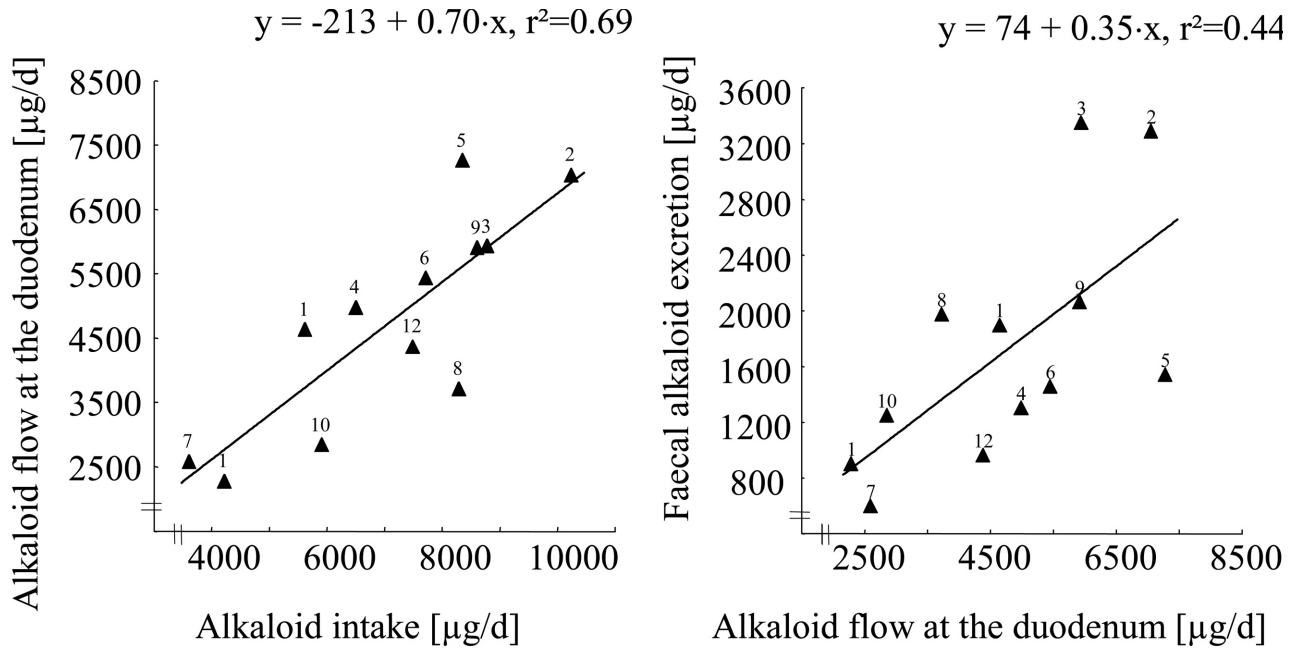

Figure 2. Alkaloid flow at the duodenum in dependence on alkaloid intake (left) and faecal alkaloid excretion in dependence on alkaloid flow at the duodenum (right) (numbers close to the symbols denote individual cows).

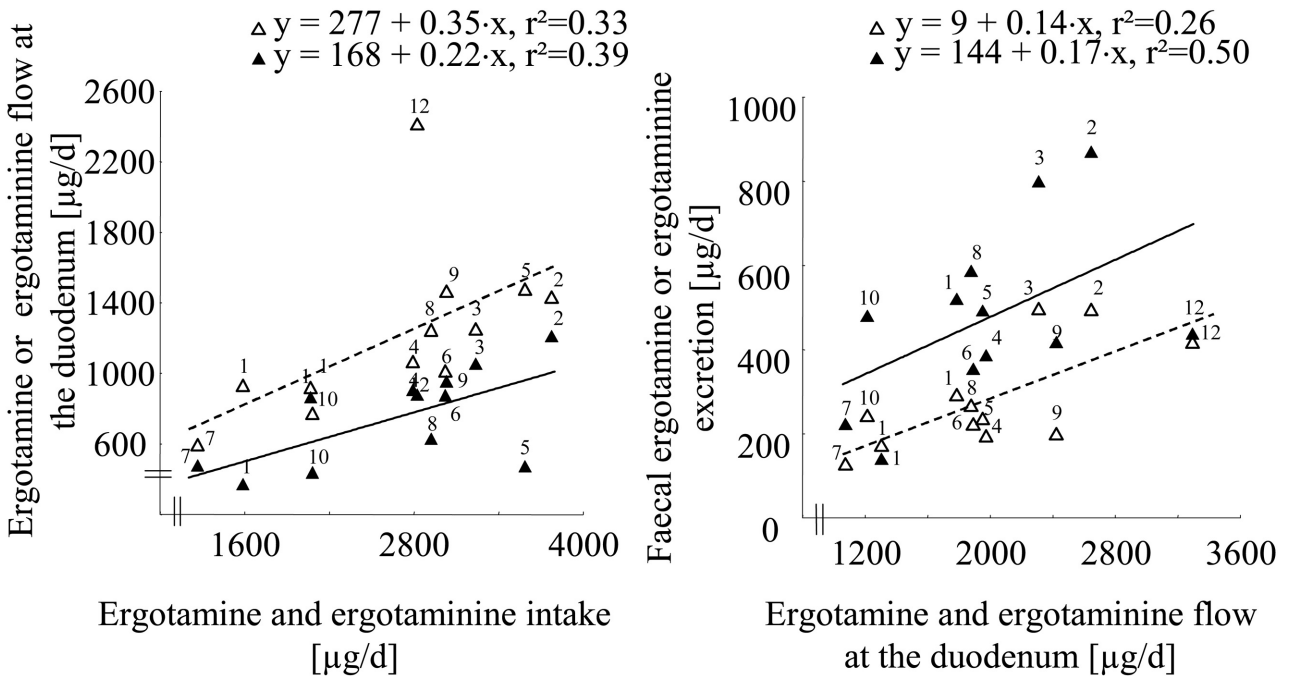

Figure 3. Ergotamine (filled triangles) and ergotaminine (unfilled triangles) flow at the duodenum in dependence on their intake (left) and faecal ergotamine (filled triangles) and ergotaminine (unfilled triangles) excretion in dependence on their flow at the duodenum (right) (Numbers close to the symbols denote individual cows).

potential effects may be attributed to the differing DMI and to the ergot supplementation of the concentrate.

The ergot used in this experiment fits into the recently reported ranges of alkaloid variation (42 – 2100 mg/kg ergot DM) in Germany [16 – 18].

Since ergot alkaloids were not detectable in the maize silage throughout the entire experimental period, the actual alkaloid exposure of the cows resulted only from the concentrate contamination and ranged from 4.1 – 16.3 µg/kg BW considering the differences in DMI and BW of the cows.

The zearalenone contamination of the maize silage of 139.4 ng/g DM relates to 75.6 ng/g DM of the total ration at a DM content of 88%. The European orientation value for dairy cows and calves of 500 ng/g [19] is more than six times higher than the zearalenone contamination in the current study. The deoxynivalenol contamination of the maize silage of 1858 ng/g DM corresponds to an approximate contamination of the total ration of 981 ng/g at a DM content of 88%. The contamination of deoxinivalenol is also significantly lower than the European orientation value of 5000 ng/g [19] and thus may not cause any negative effects.

4.2 Alkaloid metabolism and excretion

The fact that only 67% of the ergot alkaloids reached the duodenum leads to the question which part of alkaloid metabolism and absorption takes place in the rumen.

Studies about this subject concerning ergot alkaloids of *C. purpurea* are lacking, but there are some reports on alkaloid metabolism and absorption in the rumen of endophyte-produced alkaloids. Westendorf *et al*. [20] incubated different combinations of the pyrrolisidine alkaloids *N*-formyl and *N*-acetyl loline in ruminal fluid for 0, 24 or 48 h. Their disappearance increased over time and significant amounts of both alkaloids were metabolised and converted to loline. In a second experiment he used abomasally cannulated sheep which were administered doses of 945 and 2346 mg ergot alkaloids/day (ergovaline and ergovalinine). Analysing pooled samples of abomasal fluid and faeces, he recovered 50 – 60% of the alkaloids in the abomasal contents, but only 5% in the collected faeces. From the *in vitro* experiment he concluded that alkaloids in general may be degraded by ruminal fermentation. But as the *in vivo* recoveries in abomasal fluid and faeces of the indicated pyrrolisidine alkaloids were much lower than those of the ergot alkaloids, a relative stability of the ergot alkaloids towards ruminal bioconversion might be assumed [20]. Nevertheless an absorption by the ruminal tissue might be presumed which agrees with the results of Hill *et al*. [21], who placed sheep ruminal and omasal tissues in parabiotic chambers. He found the ergot alkaloid transport to be an active process and measured a much higher transport potential for lysergic acid and lysergol than for the ergopeptines (ergonovine, ergotamine and ergocryptine).

Stuedemann *et al*. [22] also postulated that ergot alkaloids must be absorbed in the forestomach system because of their rapid excretion.

Since mean recovery rates ranged between 78 and 111% in the duodenal ingesta, and between 63 and 108% in the faecal samples, it might be assumed that in the current study nearly 30% of the alkaloids were absorbed or metabolised prior to the duodenum, and nearly 70% (Fig. 2), or maybe a bit less, in the total tract, which agrees with the results of the above mentioned studies.

Ruminants are considered to be less sensitive to mycotoxins due to the potential transformations by their rumen microbes [23]. Toxic agents may be biotransformed and excreted as nondetectable metabolites. These metabolites might be degraded and thus less toxic, but might also be converted to more toxic compounds, as it is known, *e.g*., for nitrite, which is a toxic nitrate metabolite during nitrate poisoning [24]. The complexity of potential metabolites of the alkaloids has recently been reviewed by Kren [25].

But not only the microbes, also the pH-value might influence the toxicity of the ergot alkaloids. Hence, a change of pH may cause a conversion to their – inine isomers which are pharmacologically less active and thus less toxic [3, 4]. In the current study, the – inine fraction increased posterior to the forestomachs (Fig. 3), which might be caused by the low pH-values in the *abomasum*. As this conversion is reversible [3], this would also explain that the – inine fraction in the faeces is lower again (higher pH-values in the gut) (Figs. 1 and 3).

This hypothesis of pH-dependency of the alkaloid epimerisation is partially supported by accompanying *in vitro* tests (data not shown). While a stepwise increase in pH from 2 (∼pH of duodenal ingesta) to 8 (∼pH of the distal digestive tract) of a alkaloid standard solution did not result in a change of the proportion of the -inines of the sum of -ines plus -inines when measured immediately after pH-adjustment, a time and pH-dependent alteration could be demonstrated when pH was increased with time to account for the time required for ingesta transit through the gut and the therewith connected pH-increase. The proportions of ergometrinine, ergotaminine, ergocorninine and ergocristinine of the sums of the respective epimeres decreased by ∼5, 32, 21 and 22%, respectively, when a pH of ∼6 – 7 was exceeded after 24 h while the ergocryptinine proportion increased by 10% at the same time.

Urinary alkaloid excretion could be evaluated as urine alkaloid concentrations were lower than the LODs of the method used.

4.3 Ergot residues in the blood

In preceding experiments maximum doses of 9.1 μg/kg BW were fed to growing bulls over a period of ∼230 days and the alkaloid residues in the analysed blood samples were lower than the indicated LODs [26]. Also in calves exposed to maximum doses of 35.5 μg/kg BW over a period of 84 days, no alkaloid residues were detectable in the blood [27]. It has to be considered, that in these experiments, due to the feeding management the exact time between the last ergot intake and sampling of the blood was not known. Thus, most of the alkaloids might have already been excreted or metabolised into nondetectable metabolites. Ergot alkaloids are quickly metabolised within the body. Approximately 2 h. after feeding, ergotamine was found to

climax in the blood, followed by slow decreases [6]. Although in the current study the moment of sampling was exactly 2 h. after the morning feeding, no detectable residues were found. It might be suggested that analysis methods with lower LODs would be necessary to demonstrate potential alkaloid absorption.

4.4 Carry over into milk

Cunningham *et al*. [28] fed two dairy cows with 17.5 g and 24 g total alkaloids *per* day and caused lameness. No alkaloids could be detected in the milk by analysis or an animal feeding experiment, in which rats were fed the organs and tissues of the sickened cows. But it has to be kept in mind that the authors used analysis methods with rather high LODs. They used a colorimetric procedure with p-dimethylaminobenzaldehyde as a chemical assay according to an extraction method based on Kluge [29]. This method is featured for a LOD of 500 ng/g, which is much higher than the indicated LODs of the method used in the current study.

After an administration of silage contaminated with 0.1% ergot (0.132% total alkaloids in the ergot), milk samples of severely affected cows were negative for ergot alkaloids [30].

Similarly, a mean alkaloid exposure of 3 μg/kg BW over a period of 5 weeks did not cause any detectable amounts of ergot alkaloids in the milk of four dairy cows, which lead the author to the assumption that the rate of excretion is less than ten *per* cent, if there is carry over into the milk and that an accumulation of the alkaloids might be nearly excluded [31].

In contrast to these findings, Parkheava (1979) (cited by Wolff *et al.* [31]) published a carry over of ergot alkaloids into the milk of dairy cows not associated with any apparent toxicosis symptoms after an ergot exposure of ∼125 mg ergot/kg BW. This is ∼100 mg ergot/kg BW more than in the current study (≈25.6 mg ergot/kg BW). The highest alkaloid exposure in the present experiment was 16.3 μg/kg BW which is closer to the experimental conditions described by Wolff *et al.* [31]. Thus the fact that no alkaloids could be detected in the milk confirms the earlier published results.

4.5 Conclusions

A carry over of ergot alkaloids into the milk can be excluded in dairy cows fed a diet containing ergot when the daily alkaloid intake of ∼16 μg total alkaloids/kg BW is not exceeded. Moreover, the hypothesised effect of varying feed intakes on metabolism and carry over of alkaloids into milk could not be evaluated conclusively because of undetectable alkaloid residues in blood, milk and urine. Therefore, future studies should use analytical methods characterised by lower LOQs than those of the present experiment. For example, by using a LC-MS-MS method the LOQs varied between 0.17 and 2.78 μg/kg depending on matrix and alkaloid species [32] and are ∼2 – 59-fold lower than those of the present experiment.

Approximately 67% of the alkaloids fed were recovered in the duodenal ingesta, and only ∼24% of them were excreted with the faeces. Further research is necessary to clarify metabolisation and absorption rates of the alkaloids in the ruminant in more detail.

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The authors have declared no conflict of interest.

5 References

[1] Landes, E., Mutterkorn in Futtermiteln für Rinder, *Übers. Tierernährg.* 1996, *24*, 92 – 101.

[2] Seeling, K., Lebzien, P., Dänicke, S., Spilke, J., *et al*., Effects of level of feed intake and *Fusarium* toxin contaminated wheat on rumen fermentation as well as on blood and milk parameters in cows, *J. Anim. Physiol. Anim. Nutr.* 2005, *90*, 103 – 115.

[3] Wolff, J., Mutterkorn in Getreide und Getreideprodukten, in: Ocker, H. D. (Ed.), *Rückstände und Kontaminanten in Getreide und Getreideprodukten*, BEHR'S Verlag, Hamburg, 1992, pp. 113 – 137.

[4] Buchta, M., Cvak, L., Ergot alkaloids and other metabolites of the genus *Claviceps*, in: Kren, V., Cvak. L. (Eds.), *Ergot: The Genus Claviceps*, Harwood academic publishers, Amsterdam 1999, pp. 173 – 200.

[5] Rohr, K., Brandt, M., Lebzien, P., Schafft, H., Measurement of duodenal flow in dairy-cows by either total collection or spot sampling, using a special cannula, *Can. J. Anim. Sci.* 1984, *64*, 116 – 117.

[6] Kalberer, F., Absorption, distribution and excretion of [^{3}H]ergotamine in the rat. cited by: Eckert, H., *et al.* 1970. Biopharmaceutical aspects, in: Berde, B., Schild, H. O. (Eds.), 1978, *Ergot Alkaloids and Related Compounds*, Handbuch Experimentelle Pharmakologie 49. Springer-Verlag, New York 1970.

[7] Naumann, C., Bassler, R., *Die chemische Untersuchung von Futtermitteln*, VDLUFA-Verlag, Darmstadt 1993.

[8] Wolff, J., Neudecker, C., Klug, C., Weber, R., Chemical and toxicologic studies of native corn in flour and bread, *Z. Ernährungswiss.* 1988, *27*, 1 – 22.

[9] Baumann, U., Hunziker, H. R., Zimmerli, B., Mutterkornalkaloide in schweizerischen Getreideprodukten, *Mitt. Gebiete. Lebensm. Hyg.* 1985, *76*, 609 – 630.

[10] Ueberschär, K.-H., *Einfluß von Zearalenon auf Wachstum und Rückstände in den Geweben von Mastkaninchen*, VDLUFA-Kongreßband, VDLUFA-Schriftenreihe, Vol. 52, Halle/Saale 1999, pp. 425 – 428.

[11] Dänicke, S., Ueberschär, K. H., Halle, I., Valenta, H., Flachowsky, G., Excretion kinetics and metabolism of zearalenone in broilers in dependence on a detoxifying agent, *Arch. Anim. Nutr.* 2001, *55*, 299 – 313.

[12] Williams, C. H., Iismaa, O., David, D. J., Determination of chromic oxide in faeces samples by atomic absorption spectrophotometry, *J. Agric. Sci.* 1962, *59*, 381 – 385.

[13] GfE, *Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie: Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder*, DLG-Verlag, Frankfurt (Main) 2001.

[14] Universität Hohenheim (Ed.), *DLG-Futterwerttabellen Wiederkäuer*, 7. erweiterte und überarbeitete Auflage, DLG-Verlag, Frankfurt (Main) 1997.

[15] StatSoft, *Statistica for the Windows operating system, Version 7.1*, Tulsa, OK 1995.

[16] Wolff, J., Mutterkorn in Getreide; in: *Getreidekonservierung und Futterschäden durch Getreide*, Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten, Referat Landmaschinen und Energiewirtschaft, Grub 1989, Heft *30*, 28 – 36.

[17] Mainka, S., Dänicke, S., Böhme, H., Wolff, J., *et al*., Comparative studies on the effect of ergot contaminated feed on performance and health of piglets and chickens, *Arch. Anim. Nutr.* 2005, *59*, 81 – 98.

[18] Mainka, S., Danicke, S., Bohme, H., Ueberschar, K. H., Liebert, F., On the alkaloid content of ergot (Claviceps purpurea), *Landbauforsch. Völkenrode* 2007, *57*, 51 – 59.

[19] The Commission of the European Communities, Commission recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratotin A, T-2 and HT-2 and fumonisins in products intended for animal feeding, *Off. J. Eur. Union* 2006, *229*, 7 – 9.

[20] Westendorf, M. L., Mitchell, G. E., Tucker, R. E., Bush, L. P., *et al*., In vitro and in vivo ruminal and physiological responses to endophyte-infected tall fescue, *J. Dairy Sci.* 1993, *76*, 555 – 563.

[21] Hill, N. S., Thompson, F. N., Stuedemann, J. A., Rottinghaus, G. W., *et al*., Ergot alkaloid transport across ruminant gastric tissues, *J. Anim. Sci.* 2001, *79*, 542 – 549.

[22] Stuedemann, J. A., Hill, N. S., Thompson, F. N., Fayrer-Hosken, R. A., *et al*., Urinary and biliary excretion of ergot alkaloids from steers that grazed endophyte-infected tall fescue, *J. Anim. Sci.* 1998, *76*, 2146 – 2154.

[23] Hussein, H. S., Brasel, J. M., Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology* 2001, *167*, 101 – 134.

[24] Dirksen, G., Gründer, H.-D., Stöber, M., *Innere Medizin und Chirurgie des Rindes. als "Krankheiten des Rindes" begründet von Gustav Rosenberger*, Parey-Verlag, Berlin-Wien 2002.

[25] Kren, V., *Biotransformations of Ergot Alkaloids. In: Ergot, the Genus Claviceps*, Harwood academic publishers, Amsterdam 1999, pp. 229 – 266.

[26] Schumann, B., Dänicke, S., Meyer, U., Ueberschär, K.-H., Breves, G., Effects of different levels of ergot in concentrates on the growing and slaughtering performance of bulls and on carry-over into edible tissue, *Arch. Anim. Nutr.* 2007, *61*, 357 – 370.

[27] Schumann, B., Dänicke, S., Hübner, S., Ueberschär, K.-H., Meyer, U., Effects of different levels of ergot in concentrate on the health and performance of male calves, *Mycotox. Res.* 2007, *23*, 43 – 55.

[28] Cunningham, I. J., McIntosh, I. G., Swan, J. B., The nontoxicity of milk and meat from ergotized cattle, *NZ J. Sci. Technol.* 1944, *26*, 125 – 136.

[29] Kluge, H., Über den toxikologischen Nachweis von Mutterkorn, *Zeitschrift für Untersuchung der Lebensmittel* 1934, *68*, 645 – 650.

[30] McKoen, F. W., Egan, D. A., Lameness in cattle fed ergotised silage, *Ir. Vet. J.* 1971, *25*, 67 – 69.

[31] Wolff, J., Richter, W. I. F., Spann, B., *Mutterkornalkaloide in der Milch?* VDLUFA-Schriftenreihe, Vol. 40, Darmstadt 1995, pp. 521 – 524.

[32] Krska, R., Stubbings, G., Macarthur, R., Crews, C., Simultaneous determination of six major ergot alkaloids and their epimers in cereals and foodstuffs by LC-MS-MS, *Anal. Bioanal. Chem.* 2008, *391*, 563 – 576.

