Effects of facial eczema on indole flavour compounds in dairy cows

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ABSTRACT

The flavour compounds indole and skatole (3-methylindole) are characteristic of products of pasture-based production systems. The formation of these indoles by metabolism of the amino acid tryptophan in the rumen is favoured by high levels of readily-digestible protein in forage diets. However liver function is also a very important factor as indoles taken up into the animal are cleared by metabolism in the liver. An investigation of a persistent flavour taint from a dairy farm suggested a link between high milk indoles and facial eczema. As part of a genetic study, 1223 dairy cows in 4 herds in the Waikato and Bay of Plenty were screened for liver damage associated with facial eczema by measuring levels of the enzyme gamma-glutamyl transferase (GGT) in the blood. We examined levels of skatole in plasma from cows selected for high GGT (> 400 i.u. l^{-1}, 47 cows) or low GGT (< 100 i.u. l^{-1}, 43 cows) sampled from these herds. Blood indole and skatole levels overall were higher for the 'high GGT' cows compared to the 'low GGT' cows ($P < 0.001$, Mann-Whitney test). The levels of indoles in milk collected from the same cows 4 days after blood sampling on one farm showed a highly significant ($P < 0.001$) non-linear relationship between levels in milk and blood, with milk fat skatole reaching extremely high levels (> 2000 ng g^{-1}) in animals with very high blood skatole (> 20 ng ml^{-1}). As the presence of relatively few animals with such extremely high levels of indole flavour compounds in milk is likely to raise the herd mean concentration to unacceptable levels, these findings suggest that facial eczema may have significant adverse effects on milk quality as well as on production.

Keywords: milk flavour; skatole; indole; facial eczema.

INTRODUCTION

Meats and dairy products derived from animals raised on pasture have a characteristic 'pastoral' flavour. At low levels, this flavour has been described as 'full-bodied' and is an attribute valued by populations of consumers accustomed to foods from pastoral production systems. However strong 'pastoral' flavour is undesirable to consumers, particularly those more accustomed to the flavour of products from grain-finished animals, and has been described as 'off', 'animal-like' or 'grassy' (Urbach *et al*., 1972; Keen, 1998; Young *et al*., 2003).

Indole and skatole are flavour compounds which contribute significantly to 'pastoral flavour' and occur in meat (Lane & Fraser, 1999) and milk (Urbach, 1990; Lane *et al*., 2002) in higher concentrations from animals grazing pasture than animals fed a low-protein grain-based diet. They are formed from the breakdown of dietary protein in the rumen and subsequent deamination and decarboxylation of the amino acid tryptophan (Tavendale *et al*., 2005 and references cited). The indoles are absorbed through the rumen epithelium into the blood stream where the majority is removed from the blood stream by the liver (Roy *et al*., 2004). Roy *et al*. (2002) showed that the transfer of indole and skatole from the blood stream into the milk is passive, and thus they can rapidly exchange between the blood and mammary tissue without the need for an active transporter across the mammary gland. They also observed that the concentrations secreted in milk reflect the concentrations in the blood. The possibility that this process could be affected by facial eczema (FE) was raised by an investigation of a persistent flavour taint from a dairy farm affected by FE, which noticed a link between high milk indoles and facial eczema (Attwood *et al*, 2003).

Facial eczema is a disease of ruminants caused by ingestion of spores of the saprophytic fungus *Pithomyces chartarum* which grows on pasture litter. FE outbreaks occur when weather conditions suitable for rapid fungus growth (warm, humid weather and light rain) and spore production are combined with intensive grazing practices that facilitate the ingestion of spores (di Menna & Bailey, 1973). In dairy cattle, reduction in milk yield associated with liver damage and clinical disease is well known but experimental data also show that sporidesmin intakes too low to cause detectable liver damage can reduce milk volumes significantly, causing greater production losses

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than the incidence of clinical FE would suggest (Smith & Towers, 2002). The serum concentrations of the liver-derived enzyme, gamma-glutamyltransferase (GGT), are directly related to the severity of sporidesmin induced liver damage and this parameter is commonly used to monitor the incidence of sub-clinical FE (Towers, 1978; Towers & Stratton, 1978). After the initial toxic exposure, the liver begins regenerating within a few weeks and a proportion of liver function returns within 1–2 months. However, regeneration may not be complete, especially after repeated exposure to sporidesmin over several years (Smith & Towers, 2002). FE has also been reported to have adverse affects on sheep meat flavour (Kirton *et al*, 1976, 1979). As the liver is the major detoxification route for indole and skatole, a poorly functioning liver may not efficiently remove these compounds from the circulatory system, resulting in higher levels of indole and skatole in milk and meat, and adverse flavour effects.

The objective of this study was to determine a), whether FE damage to the liver of dairy cows as measured by plasma GGT levels affected concentrations of indole and skatole in blood plasma, and b), for a subset of the cows, whether such effects were observed in milk fat both immediately and in the following dairy season.

MATERIALS AND METHODS

Animals and Sampling

Four herds of dairy cows (labelled herd 5, herd 8, herd 9 and herd 11) were selected in autumn 2005, each containing at least 3% clinical cases of FE and at least 45% of cows with elevated activity of GGT. The herds were part of a study investigating genetic variation in resistance to FE in dairy cattle (Cullen *et al*., 2006). Herd 5 and herd 9 are from the Waikato and herd 8 and herd 11 are from the Bay of Plenty. Blood samples for GGT analysis were taken from every cow from each herd, regardless of clinical FE status of individual cows (a total of 1223 samples). Samples were drawn from the tail into heparinised 10 ml vacutainer tubes, which were then transported at ambient temperature to a commercial laboratory in Hamilton for GGT analysis. The blood samples were collected from herd 5 and herd 8 on 30 March 2005, herd 9 on 31 March 2005, and herd 11 on 14 April 2005. For each herd, the residual plasma from the samples from subsets of high blood GGT (> 400 i.u. l^{-1}) cows and low blood GGT (< 100 i.u. l^{-1}) cows were set aside for indole and skatole analysis (numbers of animals in Table 1).

In herd 8 the incidence of high GGT levels was very high (165 of 177 (93%) of samples elevated; 18% clinical cases). For the 20 cows selected for blood indole and skatole analysis, a 200 ml whole milk sample from each of 18 cows still in lactation was collected on 4 April 2005. These samples were chilled for transport back to Hamilton and then stored at -20 ºC. Further milk samples in the next lactation were collected from 14 of the same 20 cows on 12 October 2005.

Analytical methods

Blood samples were analysed for GGT, a non-specific indicator of liver damage by the method of Towers & Stratton (1978).

Plasma concentrations of indole and skatole (ng ml^{-1}) were measured after sample cleanup using two partitioning steps by HPLC (Claus *et al*., 1993). A 0.5 ml portion of blood plasma spiked with internal standard (2.2 ng 2-methylindole in 25 µl acetonitrile/water (75:25, v/v)) was extracted by vortexing with 2 ml diethyl ether for 30 seconds. After centrifugation (1200 *g* for 15 minutes) the samples were frozen (-20 ºC). The ether phase was decanted into tubes containing 500 µl of 0.02 M acetic acid/2-propanol (60:40, v/v; the HPLC mobile phase), to avoid losses of the volatile indoles during the subsequent evaporation of the ether phase (carried out in a water bath at 47ºC). The remaining acetic acid/2-propanol phase was filtered with a 0.2 µm filter and was analysed by HPLC-fluorescence detection.

Concentrations of indole and skatole in milk fat (ng g^{-1} milk fat) were measured by sample partitioning and HPLC (Lane *et al*., 2002). Milk fat was separated from whole milk by centrifugation at 10,800 g in a refrigerated centrifuge (4°C) for 30 minutes. A sub-sample of the milk fat was melted in a water bath (55°C) and a 100 µl aliquot was dissolved in 1 ml of hexane. Internal standard (15 ng 2-methylindole in 100 µl acetonitrile/water (75:25, v/v)) was added, the solution partitioned with 1 ml of acetonitrile/water (75:25, v/v), and an aliquot (25 µl) of the aqueous fraction was analysed by HPLC-fluorescence detection.

Statistical analysis

All data were statistically analysed using Genstat version 8.11 (Lawes Educational Trust, Oxford, UK) using non-parametric methods. The Mann-Whitney median test was used to determine if the FE status groups were different for all herds for blood and milk fat data. Logistical curves fitted to determine the relationship between blood and milk fat indoles during the autumn sampling from herd 8 were also calculated with Genstat.

RESULTS

Animals within the four herds were classified into either low or high FE status groups based on their plasma GGT concentration. In all herds there were clinical cases of FE, although clinical cases for this indole and skatole study were only sampled in herds 8 and 11. The median and range of GGT for the selected groups within each of the four herds are shown in Table 1, together with the corresponding data for indole and skatole concentrations measured in the blood of these cows. Although the background concentrations differed across the four herds, the median blood concentrations of indole and skatole were significantly higher in the high FE group than the low FE group within each herd ($P < 0.05$), and across all four herds ($P < 0.001$). Effects were observed for the indoles in herd 5 and herd 9 where plasma samples in the high FE status group were from animals having subclinical FE (elevated GGT only), as well as in herd 8 (two FE clinical animals of ten) and herd 11 (ten FE clinical animals of twelve). Not all animals in the high FE status groups showed an effect, with some overlap in the ranges of skatole and indole concentrations for the two groups in most cases.

The differentials between the low and high FE groups differed considerably between herds. The low and high FE status groups in herd 8 showed the widest differential in median plasma GGT and skatole (Table 1). The median concentrations of indole and skatole in the blood of the high FE group were 6- to 10-fold higher than those in the low FE group. Analysis of milk samples from this herd four days later showed a highly significant ($P < 0.001$) 18-fold difference in median milk fat concentrations of skatole and indole from the two FE groups, and no overlap in

TABLE 1: Median (range) of GGT, indole and skatole in blood samples from four herds of dairy cows sampled during autumn 2005. Units for GGT are i.u. l^{-1}. Units for indole and skatole are ng ml^{-1}. For each herd, median concentrations for FE status for indole and skatole differed significantly ($P < 0.05$) by the Mann-Whitney test.

Herd#	FE	N	GGT	Indole	Skatole
5	Low	15	16 (8 - 20)	0.3 (0.2 - 0.6)	0.5 (0.2 - 1.2)
	High	15	753 (422 - 2092)	0.7 (0.2 - 26.5)	0.7 (0.2 - 13.3)
8	Low	10	22 (7 - 23)	1.9 (0.4 – 4.0)	1.1 (0.3 - 2.5)
	High	10	3308 (3100 - 5090)	12.3 (2.0 -153.7)	11.9 (5.2 – 69.0)
9	Low	10	15 (11 - 21)	8.2 (0.8 - 26.4)	0.6 (0.3 - 2.2)
	High	10	2030 (1673 - 3157)	19.2 (6.4 - 44.1)	4.8 (1.6 - 22.3)
11	Low	8	25 (14 - 53)	3.5 (0.8 - 5.8)	5.3 (0.9 - 12)
	High	12	1492 (940 - 2792)	6.3 (1.4 - 80.2)	9.7 (1.1 - 118.8)

TABLE 2: GGT, indole and skatole in blood and indole and skatole in milk fat samples from herd 8 sampled during autumn and spring 2005. For each sampling date, FE status significantly affected both blood and milk fat median concentrations of indole and skatole ($P < 0.01$; Mann-Whitney test).

Sampling date	FE Status	N	Median Value (range)	
			Blood GGT (i.u. l^{-1})	
31/03/2005	Low	10	22 (7 - 23)	
	High	10	3308 (3100 - 5090)	
			Blood indoles (ng ml^{-1})	
			Indole	***Skatole***
31/03/2005	Low	10	1.9 (0.4 - 4.0)	1.1 (0.3 - 2.5)
	High	10	12.3 (2.0 - 153.7)	11.9 (5.2 - 69.0)
			Milk fat indoles (ng g^{-1})	
			Indole	***Skatole***
4/04/2005	Low	10	38 (10 - 91)	71 (28 - 142)
	High	8	700 (269 - 1528)	1332 (355 - 2415)
12/10/2005	Low	8	19 (9 - 23)	38 (12 - 60)
	High	6	40 (34 - 140)	78 (54 - 383)

concentration ranges (Table 2). In milk samples collected six months later from most of these animals during their next lactation, the milk fat concentrations of indole and skatole in the high FE group had returned to much lower levels, but the median indole and skatole concentrations of the high FE group remained significantly higher ($P < 0.01$; 2-fold) than for the low FE group.

The relationship between blood and milk fat indole (Figure 1) and skatole (Figure 2) concentrations measured in the autumn samples was fitted well by a log curve (90.7 and 88.0% of the variance, respectively).

FIGURE 1: Concentration of milk fat indole in autumn (● = high FE status; ○ = low FE status) and spring (▲ = high FE status; Δ = low FE status) vs blood indole for autumn for individual dairy cows from herd 8. The curve is fitted to the equation [milk fat indole in autumn] = a + c/(1 + EXP(-b*([blood indole] - m))) and accounts for 90.7% of the variance.

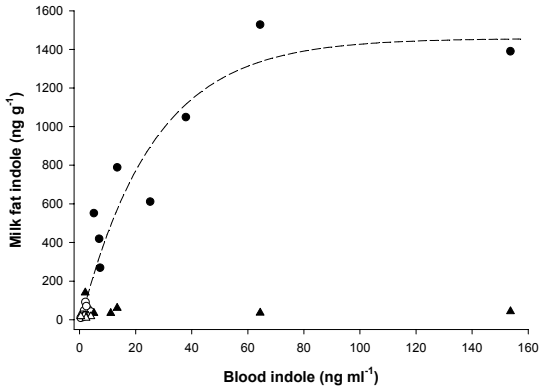
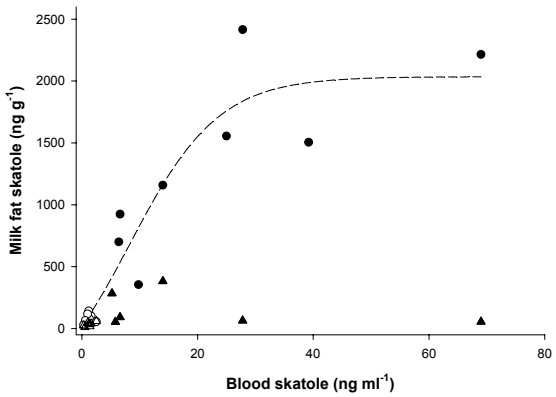


FIGURE 2: Concentration of milk fat skatole in autumn (● = high FE status; ○ = low FE status) and spring (▲ = high FE status; Δ = low FE status) vs blood skatole for autumn for individual dairy cows from herd 8. The curve is fitted to the equation [milk fat skatole in autumn] = a + c/(1 + EXP(-b*([blood skatole] - m))) and accounts for 88.0% of the variance.



DISCUSSION

This study provides clear evidence indicating that liver damage resulting from sporidesmin toxicosis can reduce the clearance of skatole and indole from the blood of dairy cows, and increase their accumulation in milk, thereby adversely affecting milk quality as well as yield.

The selection and grouping of animals from the four herds for FE status based on high/low levels of GGT in the blood, in herds with clinical cases of FE, has made possible the unambiguous detection of effects of FE on indole and skatole concentrations. As high GGT concentrations are an indicator of liver damage, and the liver is the main organ for the metabolic processing/detoxification of indoles, the higher concentrations of indole and skatole observed in the blood of the high FE status groups across all four herds indicates a strong relationship between sporidesmin-induced liver damage and reduced efficiency of the liver for catabolism of indole and skatole. However the occurrence of high blood GGT was not in every case associated with elevated blood skatole and indole, suggesting some level of liver damage can occur without impacting on indole and skatole metabolism. On the other hand this study makes it clear that skatole and indole metabolism may be impaired in animals with subclinical FE.

While the sample set analysed for effects on levels in milk fat was much smaller, the data provide clear evidence for increased accumulation of indole and skatole in the milk of animals with high FE status. The blood indole and skatole concentrations were a good predictor of milk fat concentrations four days later. Milk fat indoles were elevated above controls for all high FE status cows tested, although blood indoles for some individual animals were not particularly elevated. It is possible that concentrations of skatole and indole in milk fat are somewhat less dynamic than concentrations in blood, and provide a clearer indication of FE effects on indole and skatole metabolism.

The continuing significant margin between the median milk fat concentrations of indole and skatole within the two FE groups after six months, despite a marked overall drop, suggests a lingering effect of FE damage on liver detoxification efficiency. Thus there may be on-going effects on milk quality across seasons from animals with an FE history. These data suggest that liver function of some of the high FE status animals is still impaired, with potential adverse effects on milk quality.

As the presence of relatively few animals with extremely high levels of indole flavour compounds in milk is likely to raise the herd mean concentration to unacceptable levels, these findings suggest that FE may have significant adverse effects on overall milk quality as well as on production. A more extensive study is needed to verify the generality of these findings, and to predict the quantitative impact of the incidence of FE on the overall indole and skatole levels in milk fat from New Zealand dairy herds, and thus in the quality of New Zealand dairy products.

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