Fusaric Acid Content of Swine Feedstuffs

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A total of 48 samples of mainly Ontario-grown swine feedstuffs from the 1987–1992 crop years including whole swine feeds, dry corn, high-moisture corn, wheat, and barley were analyzed by HPLC for fusaric acid content. Average concentrations found were 35.76, 11.75, 26.37, 11.61, and 12.23 μg g^{-1}, respectively. The highest concentration found was 135.64 μg g^{-1} in high-moisture corn. Nineteen of the samples were also analyzed for deoxynivalenol and zearalenone, and average concentrations found were 3.33 and 0.35 μg g^{-1}, respectively. There appeared to be no correlation between concentrations of fusaric acid and those of other *Fusarium* metabolites. It was concluded that significant amounts of fusaric acid are present in some Canadian- and American-grown swine feedstuffs and, in light of their common pharmacological activity, that this metabolite should be included when feedstuffs are analyzed for *Fusarium* trichothecene mycotoxins.

INTRODUCTION

Fusaric (5-butylpicolinic) acid is a *Fusarium* phytotoxin produced mainly by *Fusarium moniliforme* (Burmeister et al., 1985), which is one of the more common strains of *Fusarium* fungi found in Canadian-produced grains (Neish et al., 1983). Fusaric acid is a hypotensive agent (Hidaka et al., 1969) and has a low acute toxicity when compared to other *Fusarium* metabolites. The LD$_{50}$ dose of fusaric acid for mice has been reported to be 100 mg kg^{-1} iv and 80 mg kg^{-1} ip (Hidaka et al., 1969). Fusaric acid is, however, pharmacologically active, and acute doses can elevate brain tryptophan and serotonin concentrations (Chaouloff et al., 1986). It is also a potent inhibitor of dopamine-β-hydroxylase, a key enzyme in the regulation of the synthesis of the neurotransmitter norepinephrine (Nagatsu et al., 1970). Acute doses have also been shown to cause vomiting and lethargy in swine (Smith and MacDonald, 1991).

A difficulty in determining the potential hazard posed by *Fusarium* mycotoxin contamination of animal feeds is that naturally contaminated feedstuffs are often much more toxic than would be expected on the basis of chemical analysis (Trenholm et al., 1983). This has been described in swine fed purified and natural sources of deoxynivalenol (Forsyth et al., 1977; Friend et al., 1986). It is possible that fusaric acid may be acting synergistically with *Fusarium* trichothecene mycotoxins to increase the toxicity of contaminated feedstuffs. Dowd (1988), using a caterpillar bioassay, reported that fusaric acid could synergize the toxicity of trichothecenes. The increases in brain tryptophan and serotonin following acute dosing of swine with fusaric acid (Smith and MacDonald, 1991) are similar to those observed following dosing of swine with deoxynivalenol (Prelusky et al., 1992) and following dosing of rats with deoxynivalenol (Fitzpatrick et al., 1988) and T-2 toxin (Boyd et al., 1988; MacDonald et al., 1988).

Like fusaric acid, fumonisins are also produced by *F. moniliforme*. Purified fumonisins do not alter brain neurochemistry in the manner of fusaric acid, however (Porter et al., 1993), while naturally contaminated corn containing fumonisin did, although fusaric acid content was not determined (Porter et al., 1990).

It is not possible, however, to positively identify fusaric acid as contributing to the etiology of *Fusarium* myc-

otoxicoses in livestock without an analytical procedure for fusaric acid measurement in animal feedstuffs combined with survey data regarding the occurrence of fusaric acid in the field. The current experiments were conducted to provide such information.

MATERIALS AND METHODS

Samples. The samples analyzed were collected mainly in southern Ontario from farms of swine producers who thought they were experiencing production difficulties due to the presence of mycotoxins in feeds. Samples included whole swine feeds, high-moisture corn, dry corn, wheat, and barley. Most samples were of feedstuffs grown in southern Ontario from 1987 to 1992, although some from western Canada and the United States were included. Nineteen of the samples had previously been analyzed for deoxynivalenol, T-2 toxin, and zearalenone by the Ontario Ministry of Agriculture and Food Veterinary Laboratory Services, Guelph, ON.

Extraction Procedure. The method used was a modification of that of Matsui and Watanabe (1988). Samples were ground to uniform consistency, and 2.0 g was blended with 40 mL of 1:1 methanol/1% KH$_2$PO$_4$ (pH 3.0) at high speed for 5 min. The resulting mixture was centrifuged at 20000g for 30 min, and the supernate pH was adjusted to pH 3.0 with 2 N HCl. The supernate was then sequentially extracted three times with 50 mL of diethyl ether. The organic layers were collected and pooled using a separatory funnel and subsequently filtered. The aqueous fractions were discarded. Ether was then removed and the residue rotary evaporated to dryness under vacuum. The residue was dissolved in HPLC mobile phase before injection.

Chromatography. Compounds were resolved using a 25 cm × 4.6 mm reversed-phase C$_8$ column (Supelcosil LC-8-DB, Supelco Inc., Bellefonte, PA) coupled to a Waters 510 solvent delivery system, a Waters 481 LC spectrophotometer, and a Waters 745 data module (Waters Scientific, Mississauga, ON). The mobile phase was 7:2:1 methanol/water/1% KH$_2$PO$_4$ (pH 3.4) with a flow rate of 1 mL/min. UV detection was at 271 nm.

RESULTS AND DISCUSSION

The extraction procedure used resulted in a fusaric acid recovery of 94.38% with a standard deviation of 4.64. Extraction efficiency was determined by addition of pure standards to uncontaminated samples. Extraction efficiency was reduced significantly if the exact procedure of Matsui and Watanabe (1988) was used, although this also resulted in improved resolution of fusaric acid. Fusaric acid was identified by comparison of retention times with those of known standards and further confirmed by spiking of samples with purified compound (Figure 1).

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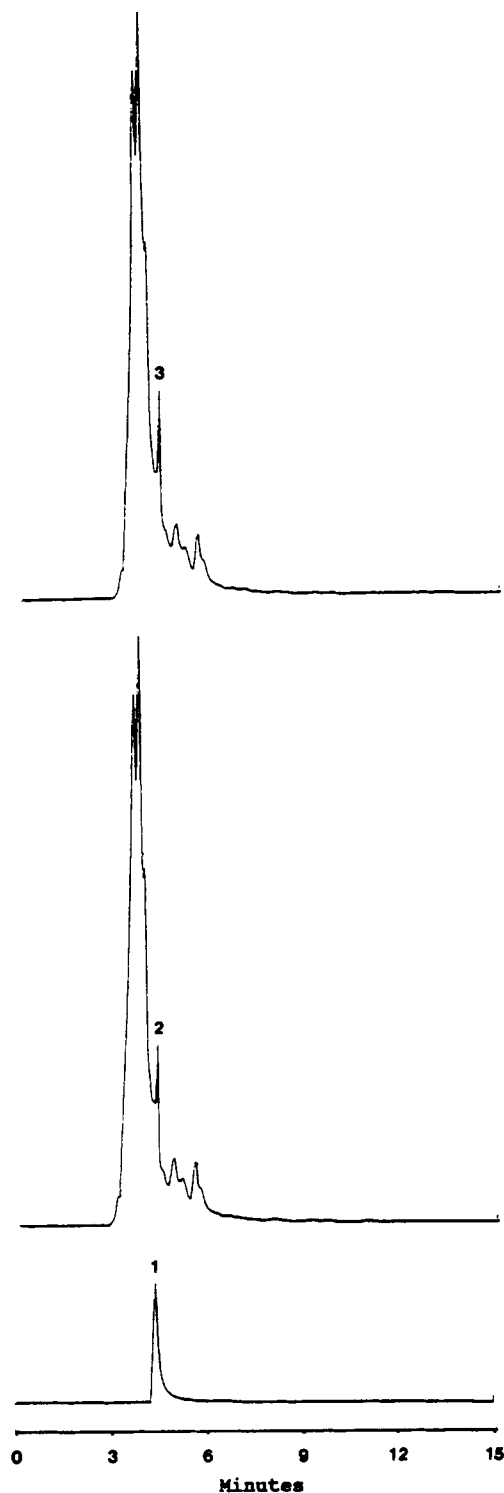*J. Agric. Food Chem.*, Vol. 41, No. 12, 1993 **2297**

Figure 1. Chromatogram of fusaric acid standard (1), an extract of a high-moisture corn sample containing fusaric acid (2), and an extract of the same sample spiked with purified fusaric acid (3).

Table I. Fusaric Acid Concentrations in Swine Feedstuffs

sample	feedstuff	fusaric acid concn, μg g^{-1}
whole feeds		
1	finishing ration	20.10
2	finishing ration	125.74
3	finishing ration	27.08
4	dry sow ration	10.32
5	finishing ration	61.16
6	finishing ration	ND[a]
7	sow feed	31.90
8	sow feed	9.74
		mean = 35.76
dry corn		
9	dry corn	4.88
10	dry corn	28.77
11	dry corn	3.01
12	dry corn	4.80
13	dry corn	3.83
14	dry corn	5.90
15	dry corn	11.00
16	dry corn	10.60
17	dry corn	ND
18	dry corn	23.00
19	dry corn	6.90
20	dry corn	ND
21	dry corn	22.10
22	dry corn	28.50
23	dry corn	13.20
24	Indiana dry corn	21.50
		mean = 11.75
high-moisture corn		
25	high-moisture corn	135.64
26	high-moisture corn	97.16
27	high-moisture corn	5.47
28	high-moisture corn	20.75
29	high-moisture corn	11.60
30	high-moisture corn	17.10
31	high-moisture corn	9.50
32	high-moisture corn	15.10
33	high-moisture corn	13.90
34	high-moisture corn	11.20
35	high-moisture corn	ND
36	high-moisture corn	ND
37	high-moisture corn	31.70
38	high-moisture corn	ND
		mean = 26.37
wheat		
39	western wheat	9.70
40	western wheat	5.80
41	western wheat	8.80
42	Ontario wheat	1.40
43	Ontario wheat	ND
44	Ontario wheat	25.40
45	Ontario wheat	11.40
46	Ontario wheat	30.40
		mean = 11.61
barley		
47	Ontario barley	11.20
48	western barley	13.25
		mean = 12.23

[a] ND, not detected (<0.77 μg g^{-1}).

The fusaric acid concentrations found in the samples analyzed are presented in Table I. Fusaric acid was found in all types of feedstuffs analyzed, although whole swine feeds and high-moisture corn tended to contain more fusaric acid than dry corn, wheat, or barley. The individual dietary components analyzed all serve as dietary energy sources. Since the fusaric acid concentration in whole swine feeds was no less than that found in the individual energy-rich components, the possibility is raised that dietary protein sources such as soybean meal may also contain significant amounts of fusaric acid. Soybean plants

have been shown to be susceptible to *Fusarium* infestation and subsequent fusaric acid contamination (Matsui and Watanabe, 1988).

The average concentrations of fusaric acid found in samples also analyzed for deoxynivalenol, T-2 toxin, and zearalenone were far higher than those found for these latter compounds. The average concentrations and ranges found were 3.33 μg g^{-1} (0.20–12.80) for deoxynivalenol and 0.35 μg g^{-1} (0.06–2.00) for zearalenone. T-2 toxin was found in only five of the samples, and the average concentration and range of these was 0.26 μg g^{-1} (ND–0.40). The average

concentration of deoxynivalenol could be enough to reduce feed intake (Pollmann et al., 1985), while this level of zearalenone would be unlikely to have any effect (Young et al., 1981). There was no obvious correlation between the concentrations of fusaric acid and the concentrations of deoxynivalenol or zearalenone. Sample 2, a highly contaminated finishing ration, contained 125.74 μg g^{-1} fusaric acid, 3.20 μg g^{-1} deoxynivalenol, and 0.33 μg g^{-1} zearalenone. Sample 25, highly contaminated high-moisture corn, contained 135.64 μg g^{-1} fusaric acid, 6.5 μg g^{-1} deoxynivalenol, and 0.47 μg g^{-1} zearlenone. The sample containing the highest concentration of deoxynivalenol (sample 12, dry corn, 12.80 μg g^{-1}) contained only 4.80 μg g^{-1} fusaric acid. Although these compounds are all *Fusarium* metabolites, they are not all produced in significant amounts by the same *Fusarium* strains.

It is interesting to note that the average concentration of fusaric acid found in high-moisture corn was more that twice that found in dry corn. Dry corn commonly contains 12% moisture, while high-moisture corn may contain twice that amount. The feeding of high-moisture corn offers economies gained through reduced cost of energy required for drying. The disadvantage is that *Fusarium* molds grow more readily and have a greater potential to produce mycotoxins when grains have higher moisture content. This is confirmed by the data presented.

One of the highest concentrations detected (125.74 μg g^{-1}) was in a sample of whole swine feed. This is lower than the equivalent acute dose of fusaric acid previously shown to produce vomiting and lethargy in pigs of about 10-kg body weight (Smith and MacDonald, 1991). Such pigs would consume about 1 kg of diet per day. At the above concentrations of fusaric acid contamination, this would be equivalent to a total daily dose of about 125 mg of fusaric acid or about 12.5 mg of fusaric acid/kg of body weight. This is about 6.25% of the acute dose (200 mg of fusaric acid/kg of body weight) used by Smith and MacDonald (1991). It is unlikely that such a dose would have overt toxic effects except in the most sensitive individuals, although more subtle behavioral changes such as reduced appetite might be observed. Any synergistic effect of trichothecenes also present would effectively increase the potential for a toxic response to this dose.

It can be concluded that significant amounts of fusaric acid are present in Ontario-grown swine feedstuffs. Considering this and the potential toxic synergism between fusaric acid and *Fusarium* trichothecene mycotoxins, analysis of fusaric acid should be included when feedstuffs are tested for mycotoxin contamination.

ACKNOWLEDGMENT

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