

## Mass spectral confirmation of oosporein in poultry rations

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Oosporein (Fig. 1), a nephrotoxic fungal metabolite, is produced by *Penicillium*, *Acremonium*, *Oospora*, and *Chaetomium* species. Unlike the mycotoxins ochratoxin and citrinin, which affect both the kidney and the liver, oosporein affects only the kidney. Oosporein has been demonstrated to cause gout and kidney nephrosis in broiler chicks and turkeys.<sup>6,7</sup> The recent development of a sensitive thin-layer chromatographic (TLC) screening method for oosporein in poultry rations<sup>9</sup> has provided a means to determine whether oosporein is present in grains and determine if it is a causative factor in renal disease in poultry.

In this paper, we report on chemical ionization (CI) mass spectral investigations of oosporein for the purpose of confirming TLC tests for the presence of oosporein in samples of poultry rations. Mass spectrometry (MS) has recently become a routine tool for confirmation and identification of various toxicants encountered by Veterinary Diagnostic Laboratories (F. Ross, personal communication). Most reports<sup>2,4</sup> on MS in diagnostic toxicology have involved the conventional ionization technique of electron impact (EI). Only in the mycotoxin area have other ionization methods been widely studied.<sup>1,8</sup> In the case of oosporein, CI provides a clearly superior alternative to EI.

For this study, oosporein was isolated in pure form from a culture of *Chaetomium trilaterale* under conditions previously described.<sup>6</sup> All mass spectra were obtained on a tandem mass spectrometer.<sup>7</sup> The EI spectrum at 70 eV by Direct Exposure Probe (DEP) was similar to that previously reported,<sup>7</sup> verifying the purified standard. Major ions included the following: m/z (relative intensity); M+ at 306 (48), 250 (10), 222 (38), 194 (12), 167 (18), 138 (28), and 83 (100).

The positive ion CI (PCI) spectrum was obtained using methane reagent gas, with source temperature of 150 C, 70 eV ionizing voltage, and DEP introduction. Major ions included the expected (M + 1)<sup>+</sup> at 307 (100) with fragment ions of 279 (18), 222 (5), 131 (7), and 83 (9) in addition to the reagent gas adduct ions at 321 (5) {M + 15}<sup>+</sup> and 335 (10) {M + 29}<sup>+</sup>.

Methane negative ion CI (NCI) yielded the most interesting results. In addition to the M- at 306 (48), (M + 2)<sup>-</sup> at 308 (100) was present. The existence of (M + 2)<sup>-</sup> is most likely due to reduction of one of the quinones to hydroquinone under the electron capture conditions of the reagent gas. Other major negative ions included 290 (31), 274 (4), and 154 (3), in addition to the reagent gas adduct ions at 320 (10) {M + 14}<sup>-</sup> and 334 (8) {M + 28}<sup>-</sup>. The fragment ion of m/z 290 further supports the existence of a hydroquinone. The

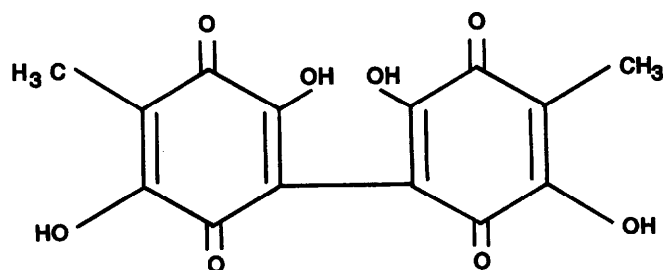


Figure 1. The structure of oosporein, 3,3',6,6'-tetrahydroxy-2,2'-dimethyl-5,5'-bi-p-benzoquinone.

m/z 290 is most likely due to the loss of H<sub>2</sub>O from the (M + 2)<sup>-</sup> as opposed to the unlikely loss of 16 from the parent M- (m/z 306). Further proof of the hydroquinone was obtained from the MS/MS daughter spectra of 308<sup>-</sup> which included 290. Tandem MS of 306<sup>-</sup> did not show a daughter ion of 290 or 288, which could have resulted from loss of H<sub>2</sub>O. Additionally, a neutral loss MS/MS<sup>5</sup> experiment showed only 308 to undergo a neutral loss of 18, not 306. Major daughter ions of 306<sup>-</sup> included 306 (100), 278 (7), 250 (4), 206 (9), 193 (11), 166 (4), and 152 (42).

Both PCI and NCI spectra are easily obtained using the MS/MS operated in the pulsed positive ion negative ion (PPINICI) mode. Alternate negative PCI and NCI spectra are produced within a single data acquisition. The combination of PCI (M + 1)<sup>+</sup> 307 with NCI M<sup>-</sup> (306) and (M + 2)<sup>-</sup> 308 provides unique criteria for the confirmation of oosporein in poultry rations. This technique has been successfully applied to extracts of feed containing 5 ppm oosporein. Extracts of blank corn and poultry ration were found to contain no spectral interferences.

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## Ovine and bovine abortion associated with *Fusobacterium nucleatum*

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In 1978, Berkhoff<sup>1</sup> commented on the dearth of knowledge concerning of anaerobic bacteria other than clostridia in diseases of veterinary importance. Although anaerobic culture methods and media have been improved, reports of research on animal diseases caused by anaerobic bacteria are still scarce. Investigation into the involvement of anaerobes in animal abortions appears to be particularly neglected. The few surveys of anaerobes in veterinary clinical specimens usually have not included aborted fetuses,<sup>2,4,11-13</sup> and routine diagnostic procedures to determine the causes of abortion in food animals usually do not include anaerobic culture.<sup>5,6</sup> A search of the literature revealed a single report of an anaerobic bacterium associated with a bovine abortion (Sit ME, Reddy CA: 1985, Abstr Conf Res Workers Anim Dis #181). Recently a nonclassified flagellated anaerobic or microaerophilic bacterium has been implicated in ovine abortion.<sup>7,8</sup>

During the last 2 years, we have isolated, in pure or nearly pure culture, an anaerobic bacterium from the abomasal contents of 5 aborted lambs and 2 aborted calves. The organism was identified by API system 28<sup>a</sup> as *Fusobacterium nucleatum* or *Fusobacterium necrophorum*. Gas chromatography revealed that it did not convert lactate to propionate. It did not produce gas in peptone-yeast-extract agar deep stab and, on initial isolation, it grew in the presence of 6% oxygen. It was thus identified as *F. nucleatum*.<sup>10</sup>

In each case, there were lesions indicating an infectious condition. The lesions included suppurative bronchopneumonia, peribronchiolar or perivascular lymphoid hyperplasia or both, focal gliosis, and necropurulent placentitis. The lesions were not pathognomonic for the specific infection, and all the lesions were not present in every case.

Other diagnostic procedures including aerobic and microaerophilic bacterial culture; virus isolation attempts; fluorescent antibody tests for leptospira, pestivirus, and bovine herpesvirus- 1; examination of Gimenez-stained placental smears for Chlamydial elementary bodies; and serologic examination of ovine fetal serum for toxoplasmal antibodies failed to reveal another cause for the lesions or the abortions.

The herd histories submitted with the cases were not com-

plete. However, all the indications were that *F. nucleatum* caused only sporadic abortion.

Diagnostic success on bovine abortions is, at best, usually less than 50%. Because common epidemic forms of ovine abortion are relatively easy to diagnose, diagnostic success may exceed 60% for sheep. However, in both species, lesions indicating an infectious condition are frequently present in the fetus or fetal placenta when no significant infectious agent can be demonstrated by the means usually employed.<sup>6,9</sup> Because anaerobes have not been incriminated often in ovine or bovine abortions, we at the South Dakota Veterinary Diagnostic Laboratory have not routinely cultured abortion specimens anaerobically. *Fusobacterium nucleatum* was discovered in several abortion specimens because it tolerates -6% oxygen, at least for a short time,<sup>10</sup> and therefore it grew initially in microaerophilic culture.

There is evidence of the involvement of a nonclassified flagellated anaerobe in ovine abortion.<sup>7,8</sup> Now there is diagnostic evidence that links *F. nucleatum* with bovine and ovine abortion. These results indicate that routine anaerobic culture of abortion specimens might contribute to the success of abortion diagnosis.

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