Abortifacient Effects of a Unique Class of Vasoactive Lipids from *Pinus ponderosa* Needles[1,2]

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ABSTRACT: *Pinus ponderosa* needle (PN) ingestion by late pregnant cows results in decreased uterine blood flow, premature parturition, and retained placentae. Further, plasma from PN-fed cows increases caruncular arterial tone (i.e., induces prolonged contraction) in an isolated perfused bovine placentome. A novel class of vasoactive lipids was isolated and identified using a bovine placentome assay-guided fractionation of CH_2Cl_2 extracts of PN. Placentome perfusion tests indicated that 1-12-dodecanedioyl-dimyristate (14-12-14) was the most potent of the PN lipids for increasing caruncular arterial tone. Late pregnant guinea pigs (GP) were used to evaluate the abortifacient activity of these vasoactive lipids. In Study 1, on d 50 of gestation, part of the control diet was replaced with chopped PN (Diet A) or chopped PN subjected to sequential extraction with diethyl ether (Et_20; Diet B); Et_20 and CH_2Cl_2 (Diet C); and Et_20, CH_2Cl_2, and methanol (Diet D). The GP on Diets A and B exhibited shorter ($P < .01$) gestation lengths and reduced ($P < .01$) pig birth weights than GP on the control diet or Diets C and D. Further, only GP on Diets A and B exhibited retained placentae. In Study 2, on d 50 of gestation, part of the control diet was replaced with chopped PN that had been subjected to exhaustive CH_2Cl_2 extraction and then infiltrated with either CH_2Cl_2 alone (Diet E), CH_2Cl_2 containing 14-12-14 (Diet F), or CH_2Cl_2 containing isocupressic acid (Diet G); then solvents were evaporated. The GP consuming Diet F had shorter ($P < .05$) gestation lengths and reduced ($P < .05$) pig birth weights than did GP consuming Diets E or G. The GP consuming Diet F also exhibited a high incidence of retained placentae. These data provide evidence that a unique class of vasoactive lipids in PN exhibit abortifacient activity in guinea pigs.

Key Words: Lipids, Parturition, Placental Retention, Guinea Pigs

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Introduction

Ingestion of *Pinus ponderosa* needles (**PN**) by late-pregnant beef cows consistently results in the premature delivery of weak calves and associated retained fetal membranes (Stevenson et al., 1972; James et al., 1977; Short et al., 1987; James et al., 1989; Christenson et al., 1992a). Short et al. (1992) also demonstrated that the sensitivity of cattle to PN-induced early parturition increased with advancing stage of pregnancy and that PN were not directly cytotoxic to calves, whose survival was dependent on their maturity at birth. Short et al. (1992) reported that there were marked species differences in susceptibility to PN-induced early parturition: cattle and bison were susceptible, but sheep and goats were not.

Jensen et al. (1989) and Stuart et al. (1989) failed to confirm any pathologic organism as the cause for

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the PN-induced premature parturition, and they were unable to find any specific lesions in dams or calves. However, Stuart et al. (1989) did observe histologic evidence of a profound constriction of the caruncular arterial bed at necropsy of these cows. Christenson et al. (1992b) reported the presence of a factor(s) in the plasma of PN-fed cows that induced prolonged and sustained contraction (i.e., increased tone) of the caruncular artery in the isolated perfused bovine placentome. Further, Christenson et al. (1992a) confirmed that late pregnant cows consuming PN experienced a progressive decline in uterine blood flow culminating in the birth of weak premature calves and exhibiting retained placentae.

Using placentome-guided fractionation, it was determined that all the vasoconstrictor activity in PN was elicited by a novel class of lipids dominated by the presence of alkanediols esterified with myristic and(or) lauric acids that were contained in a methylene chloride (CH_2Cl_2) extract of PN (Al-Mahmoud, 1994). This study was conducted to confirm the abortifacient activity of these vasoactive lipids in late-pregnant guinea pigs.

Materials and Methods

Animals

All procedures involving guinea pigs were approved by the Iowa State University Committee on Animal Care and were conducted in accordance with National Institutes of Health (**NIH**) regulations concerning the care and use of experimental animals. These studies were conducted with late-pregnant guinea pigs; we had previously determined that they are sensitive to the abortifacient effects of PN ingestion (S. P. Ford, unpublished observations). Guinea pigs were chosen because of their relatively long gestation period (≈70 d), their large, precocious offspring, and their fermentation-type (cecal) digestive system (Hagen and Robinson, 1953; Reid, 1958).

Study 1. Pinus ponderosa needles were chopped using a Fitzpatrick Model D Hammer Mill (Fitzpatrick Co., Elmhurst, IL) at medium speed with knives forward and fitted with a #8 mesh stainless steel screen. For Soxhlet extraction, 500-g batches of milled PN were exhaustively and sequentially extracted in a Soxhlet apparatus (Figure 1) with 2.5 L each of diethyl ether (Et_20), CH_2Cl_2, and methanol (CH_3OH) (Al-Mahmoud et al., 1995). Guinea pig diets consisted of unextracted PN (Diet A), Et_20 extracted PN (Diet B), Et_20 and CH_2Cl_2 extracted PN (Diet C), and Et_20, CH_2Cl_2, and CH_3OH extracted PN (Diet D). Extracts were filtered and concentrated under reduced pressure and triturated into bovine plasma with a glass mortar and evaluated for vasoactivity in a placentome bioassay as previously described (Al-Mahmoud et al., 1995). Further, Al-

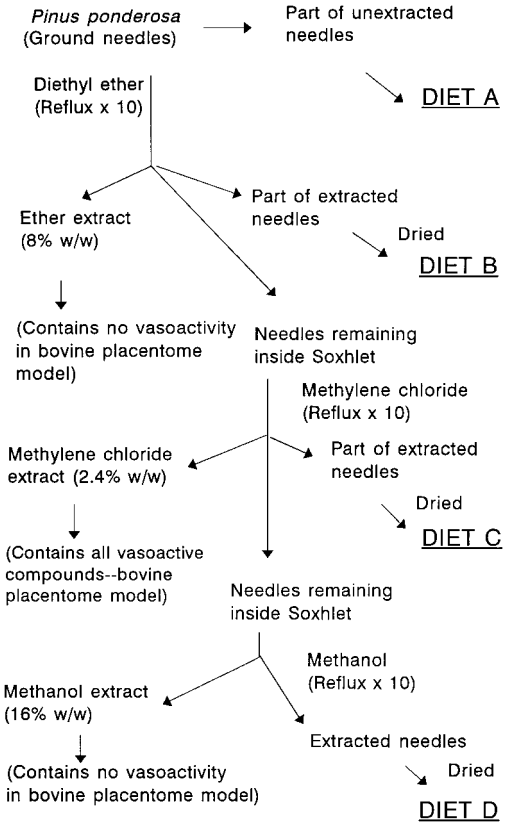

Figure 1. Soxhlet extraction scheme.

Mahmoud et al. (1995) reported that plasma containing the CH_2Cl_2 extract stimulated marked increases in tonic contractions by the caruncular artery supplying a perfused bovine placentome, but the other two extracts (Et_20 and CH_3OH) elicited no vasoactivity. These data suggest that uterine vasoconstrictive factor(s) would be present in Diets A and B, but absent in Diets C and D. This study was conducted to determine the effect of removing the CH_2Cl_2 soluble vasoactive factors from PN on its abortifacient activity.

Female guinea pigs (n = 25) were continuously housed with males and fed a commercial pelleted diet (Teklad Guinea Pig Diet 7006; Harlan Teklad, Madison, WI) and chopped alfalfa hay ad libitum. Fresh water supplemented with ascorbic acid (1 g/4 L) was also supplied ad libitum throughout the studies. Females were observed daily for estrus and the presence of a vaginal plug as previously described (Stockard and Papanicolaou, 1919). The presence of a vaginal plug was considered as d 0 of pregnancy. Beginning on d 43 of pregnancy, all guinea pigs were housed individually and limit fed 20 g of the commercial pelleted diet plus 10 g of chopped alfalfa hay (Control Diet) daily through d 49. From d 50 of pregnancy, females were randomly assigned in equal numbers (n = 5) to receive the Control Diet or an experimental diet (A, B, C, or D). Experimental diets

were identical to the Control Diet except that a 5-g portion of the chopped alfalfa was replaced with 5 g of chopped PN that had been previously subjected to Soxhlet extraction. Data from 22 guinea pigs that consumed ≥90% of all experimental diets each day for the duration of the study were included. Three guinea pigs were removed from the study for failing to consume at least 90% of their diet each day: Control Diet (n = 1), Diet B (n = 1), and Diet C (n = 1). After d 59 until farrowing or from the day of abortion if it occurred during PN feeding, all guinea pigs were fed pellets and alfalfa hay ad libitum. At farrowing or abortion, the weights of pigs and placentae (if present) were determined. Day 50 of gestation was chosen as the time to initiate the feeding of experimental diets because it was determined from preliminary trials to be the first day that PN feeding would consistently result in the pregnant females farrowing prematurely. The 10-d duration of PN feeding was determined from preliminary studies to be sufficient to induce premature farrowing in all females.

Study 2. Al-Mahmoud et al. (1995) found that a CH_2Cl_2 extract from PN contained a mixture of new structural classes of vasoactive lipids dominated by the presence of alkanediols esterified with myristic and(or) lauric acid (Al-Mahmoud et al., 1995). The most potent of these lipids in increasing caruncular arterial tone in the perfused bovine placentome was 1-12-dodecanedioyl-dimyristate (**14-12-14**; Figure 2a). This compound, when perfused through the bovine placentome at 10 μg/mL, increased caruncular arterial tone profoundly to the point of complete arterial occlusion (Al-Mahmoud, 1994). Due to its potency in increasing caruncular arterial tone, 14-12-14 was synthesized in large enough quantities for a guinea pig feeding trial. In addition, the diterpene, isocupressic acid (Figure 2b), found in PN and bark and reported to induce early parturition in beef cows (Gardner et al., 1994) was also evaluated. We were able to extract and purify sufficient isocupressic acid from Ponderosa pine bark to test in our guinea pig model. The isocupressic acid used in these studies was greater than 99% pure based upon spectral and chromatographic analyses. In our placentome perfusion bioassay, purified isocupressic acid at doses as high as 1 mg/mL failed to alter caruncular arterial tone (S. P. Ford, unpublished observations).

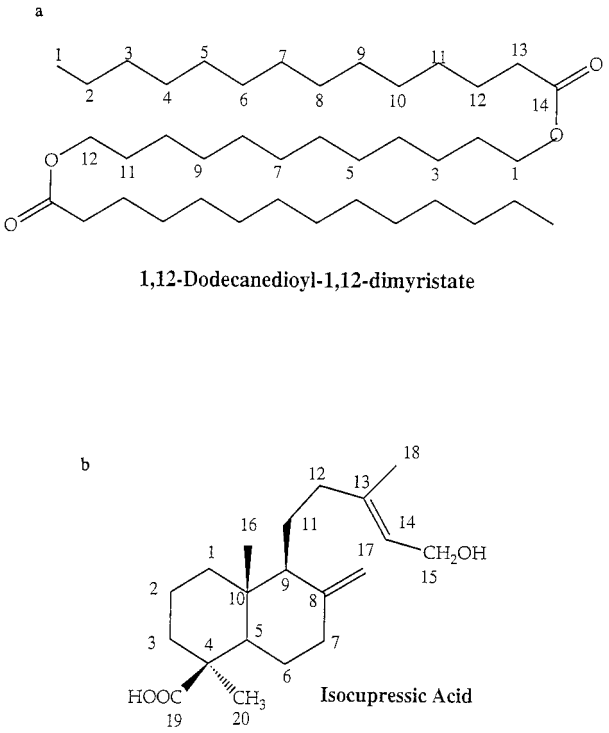

Figure 2. Chemical structures of (a) 1-12-dodecanedioyl-1,12-dimyristate and (b) isocupressic acid.

Synthesis and Characterization of 1,12-Dodecanedioyl Dimyristate for Use in Study 2

A mixture of 68.5 g (.3 mol) of myristic acid, 20.2 g (.1 mol) of 1,12-dodecanediol, 20 g of activated Dowex-50W, ion exchange resin (H^+), and 550 mL of benzene was refluxed overnight in a 1-L round bottom flask. Thin layer chromatographic analysis (CH_2Cl_2/C_6H_{12}/CH_3CN, 20:5:.1 vol/vol/vol) indicated that the reaction was incomplete. A sample of 100 mg of *p*-toluenesulfonic acid was added to the reaction mixture, and refluxing was continued overnight again until the reaction was completed. The mixture was filtered through sintered glass, the filtrate was passed directly through a dry flash chromatography column (4 × 60 cm), and compounds were eluted with benzene while fractions of 200 to 250 mL each were collected. The first three fractions, when concentrated to dryness, gave 62 g of product identified as 14-12-14 (silica gel GF_{254} thin-layer chromatography, R_f .5, in solvent system CH_2Cl_2/C_6H_{12}/CH_3CN, 20:5:.1 vol/vol/vol); mp 55 to 56.5°C; infrared (**IR**), 1,735.1 cm^{-1} and no free COOH or OH bands; proton nuclear magnetic resonance (**^{1}H-NMR**; 360 MHz, $CDCl_3$) δ, 4.1 (t, 4H, 7Hz, -OCH_2-), 2.29 (t, 4H, 7Hz, -CH_2-C00-), 1.61 (m, 8H), 1.26 (br s, 56H, -CH_2-), .88 (t, 7 Hz, 6H, -CH_3); carbon nuclear magnetic resonance (**^{13}C-NMR**; 90.15 MHz, $CDCl_3$) δ 174 (C00), 64.4 (-$CH_2$0), 34.43, 31.93, 29.69 to 29.123 (numerous unresolved signals), 28.7, 25.98, 25.06, 22.72, 14.13 (CH_3); low-resolution fast atom bombardment (**LRFAB**), 3-nitrobenzyl alcohol m/z 623.68 (88.27%, M+1)$^+$ for ($C_{40}H_{78}0_4$ + H^+ = 623.59), 413.47 (10.58%, M^+ -$C_{14}H_{27}$0), 229.28 (100%, $C_{14}H_{29}0_2$), 211.26 (81.87% for $C_{14}H_{27}$0).

Extraction and Isolation of Isocupressic Acid from Pinus Ponderosa *Bark for Use in Study 2*

Isocupressic acid was isolated from *Pinus ponderosa* bark, collected in Montana in June 1994 by modifica-

tions of published procedures (Gardner et al., 1994). The bark (500 g) was milled in a blender, exhaustively extracted with CH_2Cl_2, which was concentrated to give 54.8 g of a dark brown viscous oil. A 35.4-g sample of the extract was dissolved in CH_2Cl_2 (300 mL) and stirred with .75 *M* NaOH (320 mL) for 24 h at room temperature. The CH_2Cl_2 solution was drained and concentrated to yield yellow oil (9.9 g). The aqueous alkali fraction was acidified to pH 2 with 1 *N* HCl and extracted five times, each with 300 mL of CH_2Cl_2. The combined extracts were concentrated to give 21.6 g of dark brown oil, which was further fractionated by flash column (3.5 × 32 cm) chromatography over 40 μm of silica gel (J. T. Baker; Philipsburg, NJ) eluted under N_2 pressure (approximately 493 g/cm^2). Similar TLC fractions (3:2 hexane/Et0Ac) were subjected to repeated column purification to afford pure isocupressic acid (2.88 g). As previously stated, the isocupressic acid used in these studies was greater than 99% pure based on spectral and chromatographic analyses. The structure was confirmed as isocupressic acid from its optical rotation (Shimizu et al., 1988), mass spectra, and ^{1}H-NMR and ^{13}C-NMR spectra (Fang et al., 1989; Gardner et al., 1994).

Identification of Isocupressic Acid

The $C_{20}H_{32}O_3$, is a colorless oil, $[\alpha]_D$ + 53.4^0 (c = 1.0, $CHCl_3$) (lit.[1] $[\alpha]_D$ + 52^0 C = .94, $CHCl_3$); ^{1}H-NMR (360 MHz, $CDCl_3$, δ): 5.38(1H, t, J = 6.84 Hz, H-14), 4.84(1H, s, H-17), 4.52(1H, s, H-17), 4.15(2H, d, J = 7.2 Hz, H-15), 1.67(3H, s, H-18), 1.23(3H, s, H-20), 0.60(3H, s, H-16); ^{13}C-NMR (90.15 MHz, $CDCl_3$, δ): 183.65(C-19), 147.96(C-8), 140.48(C-13), 122.99(C-14), 106.47(C-17), 59.37(C-15), 56.36(C-7), 55.58(C-9), 44.21(C-4), 40.47(C-10), 39.16(C-1), 38.75(C-12), 38.43(C-7), 37.98(C-3), 29.01(C-18), 26.09(C-6), 21.99(C-11), 19.93(C-2), 16.36(C-16), 12.80(C-20), CIMS(CH_4) m/z(%): 407[M + H + TMSi + methyl ester]$^+$ (2), 391(10), 345(10), 317(100), 257(62); EIMS m/z(%): 406 [M + TMSi + methyl ester]$^+$ (1.1), 391(3.6), 316(15), 301(18.6), 241(35), 189(22.9), 121(100); IR $\mathbf{V}_{max}$ cm^{-1}: 3,400 to 3,000 (OH), 2,850 to 2,950 (aliphatic CH), 1,680 (C = O), 1,640 (C = C), 890(= CH_2).

Chemical Separation Techniques

Equipment. Melting points were recorded with a Thomas Hoover melting point apparatus (Philadelphia, PA) and are uncorrected. Low resolution fast atom bombardment mass spectra were obtained by using direct inlet sample introduction on a VG-ZAB-HF spectrometer. Infrared spectra were obtained using Nicolet 205 FT-IR spectrometer connected with Hewlett-Packard ColorPro Plotter (Sunnyvale, CA). Nuclear magnetic resonance spectra were obtained on a Bruker NM-360 MHz spectrometer (Billerica, MA) equipped with an Aspect-2000 processor (Billerica, MA). The ^{1}H- and ^{13}C-NMR spectra were recorded at 360.134 or 90.15 using tetramethylsilane as an external standard (δ = 0).

Diet Preparation

To prepare experimental diets, 1-kg batches of PN previously subjected to exhaustive CH_2Cl_2 extraction were added to a 3-L round bottom flask, followed by 1 L of solution of CH_2Cl_2 alone or containing 14-12-14 (100 mg) or isocupressic acid (20.0 g). The solvent was evaporated using a rotary evaporator, and the PN were dried completely at room temperature in a hood for 2 d.

The guinea pig bioassay of abortifacient activity was identical to that outlined in Study 1 except that the diets were different. Experimental diets, each of which was fed to four guinea pigs, consisted of 1) 20 g of the commercial pelleted diet + 5 g of chopped alfalfa + 5 g of CH_2Cl_2-extracted PN (control); 2) 20 g of the commercial pelleted diet + 5 g of chopped alfalfa + 5 g of CH_2Cl_2-extracted PN combined with 500 μg of 14-12-14; and 3) 20 g of the commercial pelleted diet + 5 g of chopped alfalfa + 5 g of CH_2Cl_2-extracted PN combined with 100 mg of isocupressic acid. The daily dose of isocupressic acid (100 mg/guinea pig; range in body weight ≈500 to 750 g) was based on the effective abortifacient dose of isocupressic acid (200 mg·kg^{-1} of BW·d^{-1}) used by Gardner et al. (1994) for cows. The daily dose of 14-12-14 (500 μg/guinea pig) was determined to be similar to the amount of vasoactive lipids found in 5 g of PN (Al-Mahmoud, 1994).

Statistical Analyses

Data were analyzed using the GLM procedures of SAS (1985). Dependent variables analyzed included gestation length, number of pigs farrowed, and average pig weight. The independent variable was dietary treatment. Mean comparisons were performed using LSMeans (SAS, 1985). Values of *P* less than .05 were considered significant.

Results

Study 1. Guinea pigs consuming Diets A and B exhibited markedly shortened ($P < .01$) gestation lengths (59 ± 3 and 58 ± 3 d, respectively) than those consuming the Control Diet, Diet C, or Diet D, which averaged 68 ± 1, 70 ± 1, and 68 ± 1 d, respectively (Table 1). In association with reduced gestation lengths, guinea pigs consuming Diets A and B experienced a high incidence of retained placentae (100% and 75%, respectively). In contrast, none of the females consuming the Control Diet or Diets C and D exhibited retained placentae. Pig weight was reduced

($P < .01$) at farrowing in guinea pigs fed Diets A and B versus those fed the Control Diet or Diets C and D. Also of note was the high incidence of periparturient pig death experienced by dams consuming Diets A and B (>90%) as opposed to those consuming the Control Diet or Diets C and D (< 5%).

Study 2. Gestation length of guinea pigs consuming both the Control Diet (+ CH_2Cl_2-extracted PN) and the diet containing isocupressic acid exhibited gestation lengths of normal duration, which were similar to those of females fed the Control Diet in Study 1 (Table 2). In contrast, females consuming the diet containing 14-12-14 experienced a marked shortening ($P < .05$) of their gestation length in association with a high incidence of retained placentae (75%). In association with the decreased gestation length, pigs born to guinea pigs fed 14-12-14 exhibited lower ($P < .05$) birth weight and reduced viability when compared with pigs born to females consuming the other two diets.

Discussion

Results of Study 1 confirm the results of James et al. (1994) who demonstrated in cows that all the abortifacient activity is removed after CH_2Cl_2 extraction of PN. Further, these data demonstrate for the first time that 14-12-14, the most potent member of a unique class of vasoactive lipids recently found in the CH_2Cl_2 extract of PN (Al-Mahmoud et al., 1995), returned the abortifacient activity of CH_2Cl_2-extracted PN when fed to pregnant guinea pigs. In a limited trial in late pregnant cattle, however, Short et al. (1996) reported that 14-12-14 failed to return the abortifacient activity of CH_2Cl_2-extracted PN. These authors hypothesized that the lack of effect of 14-12-14 on inducing early parturition may have resulted from an alteration in ruminal metabolism and(or) an inadequate dose. The importance of uterine arterial constriction in PN-induced early parturition is suggested by the progressive decrease in uterine blood flow in late-pregnant cows throughout 8 d of PN feeding, culminating in delivery of a premature calf (Christenson et al., 1992a). Specific effects of PN ingestion on the uterine arterial vasculature were reported by Christenson et al. (1993). These researchers demonstrated that placentomes removed on d 5 of PN feeding from late pregnant cows exhibited marked increases in caruncular arterial tone (i.e., decreased arterial diameter) in vitro when compared with placentomes recovered from cows fed a control alfalfa hay-based diet.

The fact that PN consumption induces an early parturition only if cows consume PN during late gestation may relate to the differences in the rate of fetal growth as pregnancy advances. The latter part of gestation is characterized by rapidly accelerating fetal growth. The increasing nutrient demands of the fetus over this period are met predominantly through increases in uterine blood flow resulting from a progressive reduction of caruncular arterial tone (Rosenfeld, 1984; Reynolds et al., 1985; Ford, 1995). Evidence that a chronic reduction in uterine blood flow may be sufficient to initiate premature parturition was presented by Challis et al. (1989). These researchers reported that the prolonged physical restriction (Teflon clamp) of uterine blood flow during late gestation in ewes resulted in fetal distress as evidenced by a sustained elevation in fetal ACTH and cortisol. These hormones are well established as initiators of the parturient process in many mammals, including ewes (Liggens et al., 1973) and cows (Welch et al., 1973; Comline et al., 1974; Hunter et al., 1977). As previously stated, PN ingestion induces a prolonged occlusion of the uterine vasculature (Christenson et al., 1992a). It has been hypothesized that the reduced nutrient and oxygen delivery to the fetus after PN ingestion may result in a fetal-initiated early parturition (Ford et al., 1992).

Gardner et al. (1994, 1996, 1997) reported that isocupressic acid, a major product in the CH_2Cl_2

Table 1. Effects of *Pinus ponderosa* diets on reproductive efficiency in guinea pigs

Diet fed	Gestation length, d	Number of pigs farrowed	Pig weight, g	Incidence of retained placenta[a]	Weight of individual placenta, g[b]
Control Diet	68 ± 1[c]	3.0 ± .4[c]	78.62 ± 3.50[c]	0/4	none found
Diet A	59 ± 3[d]	3.4 ± .2[c]	51.69 ± 4.02[d]	5/5	4.15 ± .33
Diet B	58 ± 3[d]	3.5 ± .2[c]	39.00 ± 4.35[d]	3/4	3.31 ± 1.19
Diet C	70 ± 1[c]	3.0 ± .5[c]	84.78 ± 7.50[c]	0/4	none found
Diet D	68 ± 1[c]	3.6 ± .4[c]	73.54 ± 9.01[c]	0/5	none found

[a]Retained placenta were considered to be those delivered after all fetuses had been expelled. In all cases of retained placenta, oxytocin (2 IU) and antibiotics were administered to facilitate placental delivery and prevent infection.

[b]Guinea pigs failed to consume placental tissue if this tissue was retained for a period of time after fetal expulsion.

[c,d]Means ± SE within a column with different superscripts differ ($P < .01$).

Table 2. Effects of *Pinus ponderosa* diets on reproductive efficiency in guinea pigs

Diet fed	Gestation length, d	Number of pigs farrowed	Pig weight, g	Incidence of retained placenta[a]	Weight of individual placenta, g[b]
Control (extracted PN)	69 ± 1[c]	2.5 ± .8[c]	74.5 ± 4.3[c]	0/4	none found
14-12-14 (extracted PN)	60 ± 2[d]	3.0 ± .4[c]	45.3 ± 3.0[d]	3/4	5.8 ± 1.6
Isocupressic acid (extracted PN)	70 ± 2[c]	2.5 ± .7[c]	71.1 ± 6.0[c]	0/4	none found

[a]Retained placenta were considered to be those delivered after all fetuses had been expelled. In all cases of retained placenta, oxytocin (2 IU) and antibiotics were administered to facilitate placental delivery and prevent infection.
[b]Guinea pigs failed to consume placental tissue if this tissue was retained for a period of time after fetal expulsion.
[c,d]Means ± SE within a column with different superscripts differ ($P < .05$).

extract of PN, also induced abortion when gavaged (hand pumped with stomach tube) (Gardner et al., 1994, 1996) or injected intravenously (Gardner et al., 1997) into late pregnant beef cows. Interestingly, anaerobic incubation of isocupressic acid with cow ruminal fluid for 48 h resulted in its transformation into two metabolites, agathic and dihydroagathic acid (Lin et al., 1998). Further, both of these metabolites have been reported to increase in blood after exposure of late pregnant cows to PN (Gardner et al., 1997; Lin et al., 1998). The vasoactive and(or) abortifacient activity of these metabolites, however, have yet to be assessed. In contrast to the data of Gardner et al. (1994), oral administration of isocupressic acid failed to return the abortifacient activity of CH_2Cl_2-extracted PN in guinea pigs at a dose reported by Gardner et al. (1994) to be effective in cattle. The failure of isocupressic acid to induce early parturition in guinea pigs may result from species differences in susceptibility or sensitivity to isocupressic acid and(or) differences in the method of administration. Differences in the responsiveness of guinea pigs and cows to the abortifacient effects of isocupressic acid cannot be excluded. Data reported here, however, provide clear evidence for the similarity of these two species to the abortifacient effects of PN when PN were fed on an equivalent body weight basis. Further, the interval from the initiation of PN feeding to parturition averaged 8 to 10 d for cows (Christenson et al., 1992a; James et al., 1994) and guinea pigs (present study), and both animals gave birth to nonviable offspring and exhibited a high incidence of retained placentae. Lastly, the sensitivity of cows to the administration of isocupressic acid-laced feed placed directly into the rumen by gavage (Gardner et al., 1994) may differ from the responsiveness of guinea pigs in the present study to natural consumption. These differences may result from the stress induced by the gavage technique itself or effects on gut absorption and(or) metabolism of isocupressic acid. In this regard, Gardner et al. (1994) reported that the interval from gavage of isocupressic acid-laced feed to abortion in late pregnant cows was ≈2 d less than the interval from the gavage of PN to abortion (5.8 vs 7.7 d, respectively).

Implications

Late-pregnant guinea pigs are a relatively small and inexpensive model for studying Ponderosa pine needle-induced early parturition. Indeed, a unique group of vasoactive lipids that are present in Ponderosa pine needles seems to be necessary for Ponderosa pine needle-induced early parturition in guinea pigs. Guinea pigs may also be useful for the identification of other ingested plant toxins that are known to affect the reproductive efficiency of ruminant species.

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