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Reproductive Toxicology: Pine Needles and Plant Estrogens[1]

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INTRODUCTION

Reproductive rate or efficiency, the number of live offspring produced from a herd of a specified number each year, is the main determinate of biological and economic efficiency of a beef cattle enterprise. Reproduction is a complex and continuous process that starts before birth and continues through puberty and a series of endocrine and behavioral events that include estrous cycles, breeding, conception, gestation, parturition, and lactation. The culmination of reproduction is live offspring produced for sale or for reentering the herd as replacements. Whenever any of these events are interfered with, reproductive rate and economic efficiency will decrease. In most beef cattle operations, the goal for reproductive rate is one calf weaned each year for each female that is two years old or older, but that rate is rarely attained because of limitations and interferences in the system. Many management (primary nutrition), genetic, and disease variables will affect reproductive rate, and these variables must be taken into account in managing a profitable ranch enterprise. However, there are cases where reproduction is interfered with even when we think we have "done everything right." Such is the case when cattle consume plant products that contain compounds that interfere with reproduction; an area referred to as reproductive toxicology. Two examples that we will cover in this review are plants that contain high levels of estrogens which potentially interfere with reproduction through effects on estrous cycles and conception and needles from Ponderosa pine trees which interfere with pregnancy maintenance during late pregnancy. There are other plant toxins that affect the general well being and health of cattle, but we will zero in on these two that primarily affect reproduction.

PINE NEEDLES

Background Information. Cattle producers in the Western United States may experience significant and even catastrophic economic losses (Lacey et al., 1988) when late pregnant cattle abort after eating needles from Ponderosa pine trees. Economic losses occur because calves are born premature and do poorly or die. Affected cows always have retained placentas that, if not treated properly, may cause complications and even death of the cow. Because of these potential losses, ranchers are not able to safely use pastures that have pine trees in them during the winter,

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and this affects pasture management for the whole year.

Physiologically, the abortive response is caused by a vasoconstrictive substance (interferes with vasodilatation or blood flow) in the pine needles. This substance causes a profound decrease in uterine blood flow (Christenson et al., 1992a; 1992b; 1993). As a result the calf is stressed and induces parturition. Thus, the effect is an induced parturition rather than an abortion. We still refer to the problem as pine needle abortion because the expression is so commonly used. Nonpregnant cows, steers, and bulls are not affected.

The response becomes more pronounced as pregnancy progresses. In the first trimester of pregnancy, very few cows are affected, some will be affected in the second trimester, and almost all cows are susceptible during the third trimester, especially in the last 30 to 60 days. Cows need to eat from 3 to 5 pounds of needles for several days to induce the abortions, and the abortions will stop within 2 to 3 days after the source of pine needles is removed or the cows are removed from the source (James et al., 1989; Short et al., 1992; 1994; 1995a).

The solution to this problem depends on finding a way to block the effects after the cow eats the needles or by preventing cows from consuming needles. Altering the diet once cows have eaten pine needles has not affected their response. Dietary variables studied have included vitamins, minerals, an organic binder (bentonite), forage (hay, straw, silage, etc.), and concentrates (Short et al., 1994). We can block the induced parturition (Short et al., 1995b) by feeding a progestin (MGA) or a prostaglandin inhibitor (such as aspirin). However, these treatments only block parturition. They do not correct the underlying problem of a drastic decrease in blood flow to the uterus and calves may die in utero.

Discouraging cows from eating pine needles in the first place has been equally unsuccessful. The only dietary variable that prevents pine needle consumption is when cows are fed 25% or more com silage. Why this occurs is not known. However, feeding corn silage is not a practical solution on most farms or ranches where pine needle abortion is a problem. Low protein content of the diet will decrease pine needle consumption, but it will not prevent it. Many other dietary variables have been tested unsuccessfully.

One approach scientists use to understand biological systems is to compare form, function, and responses among species. By comparing differences in response to treatments and differences in physiology, it is often possible to determine the how and why of a biological system or phenomena. In the case of pine needle abortion, our goals were to compare response of different animal species to pine needle consumption and different species of pine for whether or not they cause the problem. Animal comparisons provide clues to physiological and metabolic mechanisms. Plant comparisons provide clues to the active components.

Research on animal species response. Effects of pine needles on different animal species were studied in feeding trials. Ponderosa pine needles were fed to late pregnant females of different species at the rate of about 25% of their diet. Bos taurus (several European breeds) and Bos indicus (Brahman) cattle, guinea pigs, bison, goats, domestic and bighorn sheep, elk, and whitetail deer were tested (Panter et al., 1987; Short et al., 1992; Bellows et al., 1996).

Blood samples were taken before and during feeding to determine physiological effects of pine needle consumption. Interval to parturition was used as an indicator of abortifacient activity.

Both cattle species had induced parturitions in response to consuming Ponderosa pine needles. Guinea pigs responded similar to cattle. The guinea pig response is now used as a bioassay for activity in pine needles (Ford et al., 1997). When pine needles are mixed with their only feed source, bison eat them and abort like cattle. However, several herds of bison in the U. S. graze in areas with Ponderosa pine trees during late pregnancy with no observed abortions or decrease in calving rates. Thus, bison may not normally eat pine needles. Other than providing an assay, these two species do not help much as a model to understand pine needle abortion because they are not different from cattle.

Table 1. Summary of animal species response to Ponderosa pine needles (PN)[a]

Species	Abort when fed PN	Plasma VC[b] activity from PN	Eat PN when grazing	Abort when grazing PN
Cattle				
Bos taurus	++	++	+	++
Bos indicus	++	?	?	?
Bison	++	?	-	na
Goats	-	+	?	?
Sheep	-/+	+	?	?
Guinea pig	+	?	na	na
Elk[c]	-	-	++	-
Mule deer[c]	?	?	++	-
Whitetail deer[c]	-	?	++	-
Bighorn sheep	-	?	?	?

[a]Response categories: + = yes, - = no, ? = no data, na = data not available and(or) are not applicable.
[b]VC = vasoconstrictive activity in a placentome perfusion assay.
[c]Data partially from Hamlin, 1979; Dusek, 1980; Nellis, 1968; Nellis and Ross 1969.

Sheep and goats are partially different from cattle in that they do not abort. However, sheep may have an increased incidence of dead fetuses at birth. Both sheep and goats, like cattle, have high vasoconstrictive activity in blood samples taken 3 or 4 days after pine needle feeding starts. This would indicate that the activity passes through their GI tract and is absorbed into the blood just as in cattle. Either the blood flow mechanisms are different or are less responsive in sheep and goats as compared to cattle. Because of this similarity, these two species also have limited usefulness in helping to understand pine needle abortion.

Elk provide an interesting model in that they inhabit areas where Ponderosa pine trees are often prevalent. They eat pine needles and have normal calving rates in the wild. In an experiment where pine needles were mixed with their diet, elk did not abort or have increased vasoconstrictive activity in plasma. How elk avoid effects of the active material from the pine needles is unknown. When ruminal contents from elk have been incubated in vitro with pine needles the vasoconstrictive activity was not present. In companion trials with ruminal contents from cattle there was vasoconstrictive activity. Ruminal contents from elk and goats were transferred to pregnant cattle before and during pine needle feeding of the cattle. Neither elk nor goat ruminal contents prevented the abortive effects of the pine needles. As we will see later, the problem may be that cattle rumen microbes may actually produce the active material; whereas, species that do not respond do not produce the active material. If this is true, then transfer of rumen microbes will not work to prevent the problem as it has been shown with other plant toxins (Hammond, et al., 1989).

It is interesting to speculate on why there may be species differences in response to pine needles. Both Bos taurus and Bos indicus cattle evolved in parts of the world where they did not have access to needles from Ponderosa pine trees. Thus they would not have needed to devise a strategy to deal with any adverse effects. On the other hand, many wild species of ungulates evolved in environments with access to these needles. Bison will abort when force-fed pine needles. They apparently evolved a strategy of not consuming them. Elk may need access to pine needles as a nutrient source at some times of the year. Thus, they developed a strategy of not producing (or inactivating) the abortifacient activity. Other species such as deer and bighorn sheep which did not abort when fed pine needles may have evolved the same or other strategies.

Research with needles from different species of trees. Most research on pine needle abortion has been conducted with needles from Ponderosa pine. All collections from Ponderosa pine trees over several years had about equal activity regardless of geographic location, age, on trees or on the ground, and fresh or weathered. Therefore, there is no variation in needles from Ponderosa pine to use as an aid in determining the active ingredient(s).

Collections from other species may be useful to identify potential differences in abortifacient response and associated differences in chemical composition. Data from other pine species would also be useful so that producers can know which of them are or are not potential problems. Pine needles were collected from various species of pine trees, dried and ground. The ground needles were fed to cows in late pregnancy at the rate of 4 to 6 lb/day. Loblolly, Lodgepole, Red, and White pine do not have detectable abortifacient activity. These four species of pine that do not cause abortions are being evaluated. This information will be used to help answer the questions regarding the active ingredient.

Research on identifying active chemicals in pine needles. Two chemical compounds or classes of compounds have been identified as the possible culprit(s) in pine needle abortion. They are isocupressic acid (ICA, Gardner et al., 1996) and vasoactive lipids (VAL, Al-Mahmoud, et al., 1995, 1997). However, the data are confusing as to which of the two or some combination is actually involved. Even though VAL are highly vasoactive, when fed to cattle the VAL did not cause abortions. A semipurified preparation of ICA obtained from PN and fed to cattle induced abortions (Gardner et al., 1996), but when we tested purified ICA in our placentome perfusion system, it did not have any vasoconstrictive activity. More recently we (Lin et al., 1997) found that when ICA is incubated with rumen fluid from cattle, ICA is converted to two compounds which may have vasoactivity. More research is necessary to sort out the meaning of these data.

Conclusions. The effects of pine needles on inducing abortions in cattle are now well documented to be primarily a problem in late pregnant cows and are manifested by a profound decrease in blood flow to the uterus, stress to the calf and premature parturition. Assays for the biological activity have been developed using pregnant guinea pigs and a late pregnant placentome perfusion system. Several species of wild ungulates (bison are an exception) do not respond to the effects of pine needles probably because of differences in ruminal metabolism, but attempts to transfer ruminal microbes from other species to cattle have not been successful in preventing pine needle effects. Two classes of chemical compounds have been identified as potential culprits in the effects of pine needles, but we do not know for sure how or if each one is actually involved. Attempts to counteract the effects of pine needles have not been successful, and only feeding corn silage has prevented consumption of pine needles. Of those species tested, needles from Ponderosa pine are the only ones that have been shown to induce the problem. Producers must still rely on management strategies to minimize the exposure of cows to needles from Ponderosa pine trees in late pregnancy.

PLANT ESTROGENS

Chemical Nature of Estrogens. Estrogens that are produced by animals are part of a class of compounds called steroids. Steroids are synthesized in animal tissue from cholesterol, and there are four main categories (estrogen, androgen, corticoid, and progestin) based on biological activity. Shown in Figure 1 is an example of a specific compound in each classification and the parent compounds, cholesterol and pregnenolone. Note that only minor changes in the chemical structure changes the biological classification. The primary estrogen in the body is estradiol-17¢, but there are others such as estrone and estriol. One of the structural components of an estrogen is the phenol ring on the left side of the molecule. Other compounds that are not steroids may have that or other structural similarities to estrogens and show estrogenic activity. Such is the case with estrogens that are shown in Figure 2. These estrogens may be synthesized as is the case with stilbestrol, or they may be produced by plants (coumestrol). To complicate matters there is another class of compounds produced by mold or fungi called mycotoxins that also may have estrogenic activity. Zearalenone and zearalenol are examples of estrogenic mycotoxins.

Role of Estrogens in the Body. Estrogens are in a general class of compounds that exert estrogenic effects in the body and were named originally when it was discovered that they cause estrus or heat to be expressed. Estrus is the set of behavioral characteristics displayed by females that include receptivity to be bred by a male just before ovulation and is the start of the estrous cycle in most female mammals (Homo sapiens being one of the main exceptions). Estrogens are produced mainly in the ovary but also are produced by the placenta in late pregnancy and by the testicles and adrenal glands. We now know that estrogens have many effects in addition to inducing behavioral estrus that include orchestrating a complex series of endocrine, neurological, and ovarian processes that lead up to sexual maturity (puberty) and continuation of estrous cycles as well as contributing to uterine and mammary development and function, parturition, and bone and muscle growth.

The effects of estrogens may be stimulatory or inhibiting and this effect may change depending on the physiological state of the animal or the concentration of the estrogen. An example of this dichotomy of effects is that estrogens are in low concentrations and inhibit gonadotropin secretion before puberty. But as puberty approaches, the concentration of estrogen increases and the effect switches from inhibition to stimulation of a cascade of events that include follicular development on the ovaries, expression of estrus, luteinizing hormone release, ovulation, sperm transport, conception, pregnancy, and parturition.

Because of the ubiquitous nature and array of effects of estrogens, it is not surprising that there are many instances where estrogens given (exogenous or external source) to animals either intentionally or unintentionally result in dramatic negative or positive effects. Examples of desired effects in human medicine would be the use of estrogens in birth control pills and treatment of postmenopausal disorders in women. Estrogens also are used beneficially in animal agriculture to improve treatments to induce and synchronize estrus and to increase rate and efficiency of gain. The effects on gain using stilbestrol were shown almost 50 years ago. Stilbestrol is not currently approved for use because of potential carryover effects with pregnant women. Modern products use the natural estrogen, estradiol-17¢, as part of implant treatments to enhance growth.

But not all effects of estrogens are beneficial. Such can be the case when animals ingest plants or plant products that contain estrogens. These compounds are called phytoestrogens, and they sometimes interfere with production and reproduction. Phytoestrogens are mainly produced by legume plants such as soybeans, clover, and alfalfa; and their production is increased by plant stress (drought, disease) and by fertilization with material (such as sewage products or some manure) that may contain estrogens or estrogen precursors.

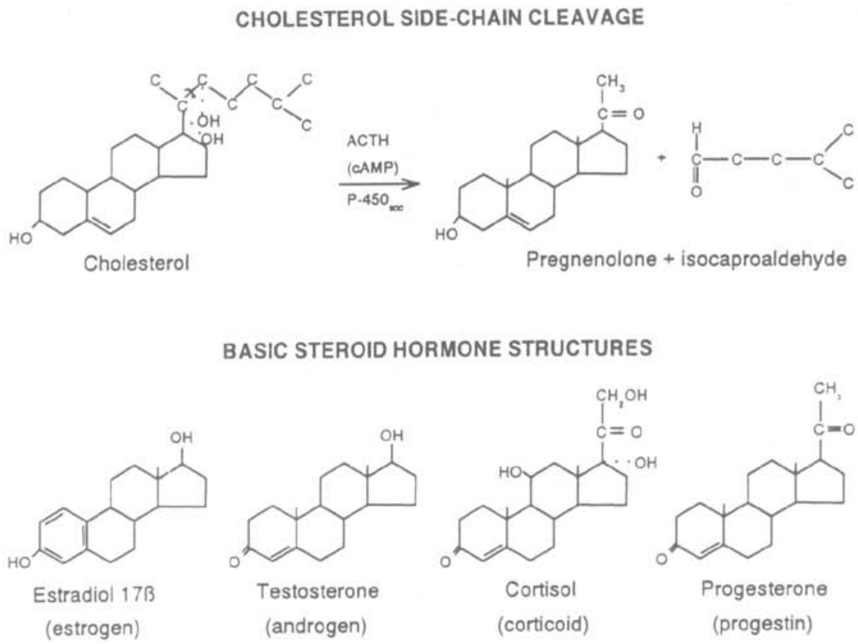

Figure 1. Chemical structure of cholesterol, pregnenolone and steroids. Cholesterol and pregnenolone are precursors of all compounds in the four classes of steroids.

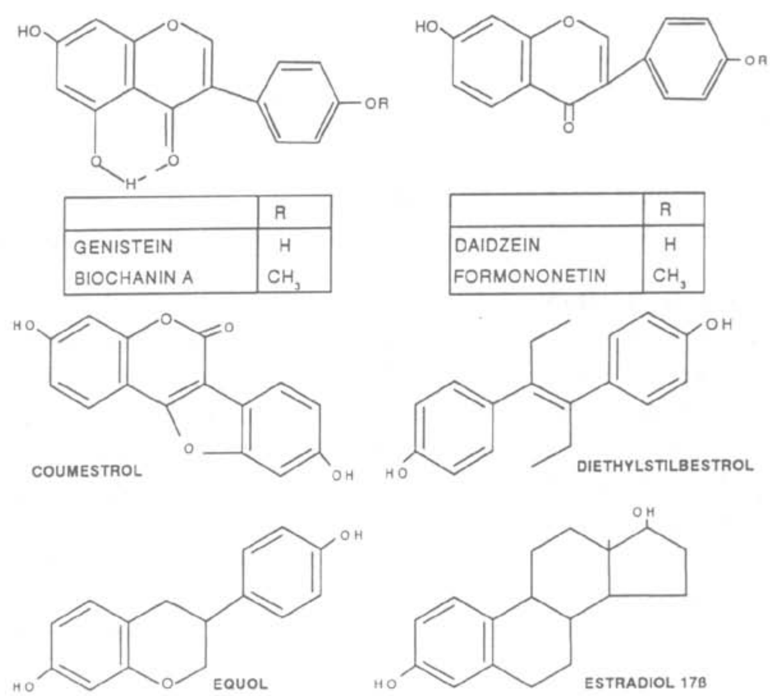

Figure 2. Chemical structure of several phytoestrogens in comparison to the steroidal estrogen, estradiol 17ß and a synthetic estrogen, diethylstilbestrol (from Shemesh and Shore, 1994).

Effects of Phytoestrogens in Sheep and Dairy Cattle. Shemesh and Shore (1994) and Adams (1995) have recently reviewed this topic, and we will rely heavily on those reviews in this discussion. Sheep and dairy cattle have been studied the most. A synopsis of that research is as follows:

1. Most research has been done in Australia, New Zealand, Israel, Europe and Southern United States, and these are the areas where most problems have been documented in sheep and dairy cattle.

2. Subterranean clover has been the most common plant with a problem of high estrogen content, but other legumes such as alfalfa, red clover, white clover, and soybeans also may have high estrogen content. New varieties have been developed with lower estrogen content.

3. Estrogen content is highest during rapid plant growth, when fertilizer is used that is high in estrogen or if the plants are stressed by drought or disease.

4. Effects in sheep range from very mild symptoms that may include temporary infertility all the way to permanent sterility.

5. Effects in dairy cattle are less severe but still may result in temporary periods of infertility. Shemesh and Shore(1994) concluded that alfalfa should not contain more than 20 ppm of estrogen (coumestrol) to avoid the problem.

6. Problems are only observed with females; no adverse effects have been found in males.

Effects of Phytoestrogens in Beef Cattle. This is the area that most of you are interested in, but unfortunately, it is the area that the least is known about. Very little research has been published to document the extent of problems with phytoestrogens in beef cattle. Most of what is known is based on work with sheep and dairy cattle and on circumstantial evidence, anecdotal information and observations of producers and agribusiness persons directly involved with nutritional and reproductive products (estrous synchronization). We will try to summarize this information, but remember, most of these conclusions are not based on experimental evidence.

7. Problems seem to be most prevalent with feedlot cattle and at times when reproduction is being intensively managed with estrous synchronization and AI.

8. Sources of estrogens could be sweet clover, alfalfa, soybean products, moldy hay, or moldy grain (remember the mycotoxins). Curing into hay may (but not always) reduce estrogen content but ensiling does not.

9. Cattle, especially those in feedlots, that are on products with estrogen (many growth stimulating implants) or products that elevate endogenous estrogen (MGA) may be more sensitive to exposure to phytoestrogens.

10. Symptoms in feedlot cattle include "bulling" or excessive riding in steers and heifers, but it is more common in heifers. Heifers will have a higher incidence of prolapse, both vaginal and rectal. Symptoms in breeding cattle may include depression of signs of estrus or just the opposite with excessive expression of estrous signs with depressed conception rates.

11. There is not a good data base for estrogen content of many of the feeds that are grazed by or fed to beef cattle. Assays are difficult to run and standardize among laboratories.

Conclusions. Effects of phytoestrogens in sheep and dairy cattle are well documented with the effects in sheep being much more severe. Management alternatives that include appropriate pasture management, selection of low estrogen varieties and feed monitoring are available to prevent these effects. Effects in beef cattle are not well documented but are probably important in many production situations. When managing beef cattle in feedlots and during the breeding season, be aware of symptoms and potential sources of estrogen - any legume pasture, hay or haylage; moldy hay or grain; or any estrogenic products that the animals may be receiving. Devise grazing, feeding and management strategies to minimize potential risks.

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