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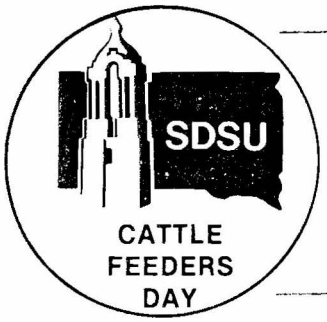

ISOLATION OF THE ABORTIVE FACTOR IN PONDEROSA PINE NEEDLES

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Summary

Chick embryos can serve as an economical means to screen pine needle extracts for possible abortive components which affect cattle grazing alpine pastures. Lipid-like extracts from pine needles did not appear to be toxic to chick embryos, while carbohydrate components were toxic at low levels. Protein components were not screened in this study.

Introduction

Although pine needles have been suspected as a cause of reproductive failure in livestock as early as 1927, pine needle abortion was not experimentally produced until 1952. Reports of suspected pine needle abortion continue throughout ponderosa pine grazing ranges of the western United States and Canada. Economic loss from aborted calves and cows lost from retained placentas has been high in the Black Hills of South Dakota during some winter periods.

The current study was initiated to develop cheaper methods to biologically screen extracts from pine needles for abortive activity and to further define components showing abortive potential.

Materials and Methods

Cold water and other solvent extractions were made from needle collections at the Arneson Ranch near Hot Springs, South Dakota. This area has a history of suspected pine needle abortions among grazing cattle. Since chicken embryos have been used to study the effects of drugs and abortive diseases, this method was selected as a possible screening procedure for pine needle extracts. Hatching eggs of the White Leghorn strain were incubated at 37.2 C for 4 days prior to injections of extracts into the air sac. The extracts were injected with a 6 mm needle and the egg was securely resealed with tape. Either distilled water or the solvent without pine needle extract served as controls. The embryonated eggs were candled every 3 to 4 days to observe the effects of the injected material on the developing embryo. Observations were continued until hatching or embryo death. At least 30 fertile eggs were used for each treatment material or treatment level.

Results and Discussion

Water extracts from pine needles showed significantly higher chick embryonic death loss than did the distilled water control, suggesting that the toxic factor is contained in a water soluble component (table 1). This confirms

previous experiments using mice and rats to biologically screen extracts. Ether extracts which contained the lipid components of pine needles showed no detrimental effect to chick embryos. In fact, a protective effect was noted (table 2). Purified water soluble, nonfibrous carbohydrates, however, significantly increased egg embryo deaths (table 3). No known toxic sugar components could be isolated from the carbohydrate fraction. The toxic component could be a complex of protein and carbohydrate (glycoprotein). At the present time, isolation of protein material having abortive potential is being attempted.

It would appear chick embryos could serve equally as well as small animals as a biological screening method for determining the presence or absence of toxic factors in pine needle extracts. Fertile eggs used in this method are convenient, relatively cheap as compared to rats and mice and require no feeding. The progress of the toxicity effect on the embryo can be followed by a simple candling procedure without injury to the embryo. Death of the embryo is easily determined by blood spots and lack of embryo movement. Its greatest value is, of course, convenience and economy. Like any preliminary screening procedure, the results would have to be confirmed by tests with the large animal concerned. Screening methods in general serve to accelerate research efforts and reduce costs.

Table 1. Effect of Treatment on Chick Embryo Mortality
Gross Pine Needle Water Extracts
Experiment 1

Treatment[a]	No. dead embryos	No. viable embryos
Distilled water	6	24
Pine needles		
10 mg/ml extract	10	20
20 mg/ml extract*	18	12
Pine needles, autoclaved		
10 mg/ml	11	19
20 mg/ml	7	23
Spruce		
10 mg/ml	7	23
20 mg/ml	7	23

[a] Two-tenths ml of each treatment solution was injected into egg air sac. Concentration is based on amount of freeze-dried extract present per ml of solution.

* $P<.01$.

Table 2. Effect of Treatment on Chick Embryo Mortality
Pine Needle Alcohol and Acetone
Extracts (Lipid Fraction)
Experiment 2

Treatment[a]	No. dead embryos	No. viable embryos
Alcohol (20 µl)	18	12
Alcohol solution (20 µl)		
5 mg/ml	16	14
10 mg/ml	17	13
15 mg/ml	19	11
20 mg/ml	20	10
Acetone (10 µl)	23	7
Acetone solution (10 µl)		
20 mg/ml	10	20

[a] Amount injected into egg air sac indicated in parenthesis. Treatment level is based on amount of freeze-dried extract present per ml of solution.

Table 3. Effect of Treatment on Chick Embryo Mortality
Purified Water Soluble Extract
(Carbohydrate Fraction)
Experiment 3

Treatment[a]	No. dead embryos	No. viable embryos
Distilled water - control	6	24
Purified water soluble extract		
5 mg/ml	15	15
10 mg/ml	16	14
15 mg/ml	15	15
20 mg/ml	10	20
Repeat		
Distilled water - control	15	45
Purified water soluble extract		
20 mg/ml	26	34

[a] Two-tenths ml of each treatment solution was injected into the egg air sac. Concentration is based on the amount of freeze-dried extract present per ml of solution.