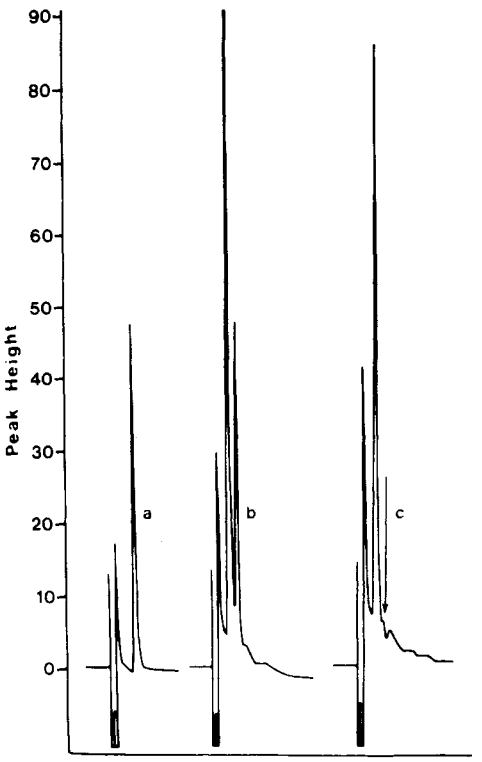

Figure 1. Chromatogram a, 2.0 ng of allidochlor (equivalent to 100 ppb) in hexane at attenuation ×8; chromatogram b, recovery from a leek check fortified at 100 ppb; chromatogram c, leek check.

detector (N-FID) rather than by microcoulometric detection using a chloride titration cell. Even though the allidochlor molecule contains only one nitrogen atom, the N-FID provided adequate sensitivity, with 4.2 ng of allidochlor giving a full-scale recorder deflection at attenuation ×8, range 1.

Analysis of the check samples showed only a small interfering peak at the retention time (3.7 min) for allidochlor (Figure 1, chromatogram c). The maximum interference from this peak, which appeared on the tail of a much larger background peak of retention time of 2.5 min, was 3–4 ppb, readily permitting a limit of detection of 100 ppb. The recovery of allidochlor from fortified leek check tissue, determined from six replicates that were analyzed at the 100-ppb fortification level, was 75.5 ± 6.3% (Figure 1, chromatogram b).

No significant differences in allidochlor residues were observed between preemergence and pre- plus postemergence applications or following a second postemergence application as residues in all samples regardless of treatment were less than 100 ppb. The registration of both pre- and postemergence applications of allidochlor for weed control in onions in Canada was based on allidochlor residues in the mature onions being less than 100 ppb (Bennet, 1983). In the present study, leeks that had been treated with similar applications of allidochlor at similar rates also had residues less than 100 ppb.

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Registry No. Allidochlor, 93-71-0.

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Allan Cessna

Agriculture Canada
Research Station
Regina, Saskatchewan, Canada S4P 3A2

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Analysis of Coumestrol, a Phytoestrogen, in Alfalfa Tablets Sold for Human Consumption

Three locally available brands of commercial alfalfa tablets were analyzed for their coumestrol content by high-performance liquid chromatography and were found to contain from 20 to 190 ppm of this phytoestrogen. The recommended dosage of the alfalfa tablets that contain 190 ppm of coumestrol would provide greater than 1.1 mg/day coumestrol. These findings raise the possibility that those who take some brands of alfalfa tablets as a dietary supplement may be unwittingly receiving an unwelcome amount of this estrogenic hormone.

The presence in forage crops of naturally occurring nonsteroidal substances with estrogenic activity has been recognized for some time (Bradbury and White, 1954). Coumestrol, a benzofurocoumarin, is the predominant plant estrogen in alfalfa (Bickoff et al., 1964). A study of the relative potencies of five estrogen-like compounds commonly found in forages—coumestrol and the four isoflavones genistein, biochanin A, formononetin, and daidzein—showed coumestrol to be 35 times more potent than the most potent of the isoflavones as measured by the mouse uterine weight bioassay (Bickoff et al., 1962). By this same assay, coumestrol was some 200 times less potent than the animal estrogen, estrone, and almost 3000 times less potent than the synthetic estrogen, diethylstilbestrol. In spite of this apparent low level of potency, high levels of phytoestrogens in forage crops fed to cattle, sheep, and other animals have been found to result in deleterious biological effects, including increased teat

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length, gestation time, and uterine weight (Bradbury and White, 1954; Braden et al., 1964; Bickoff et al., 1960). Cattle fed haylage containing as little as 37 ppm of coumestrol as their major feed show deleterious estrogenic effects (Lookhart, 1980). Sheep are even more sensitive to phytoestrogens than cattle, and because of this greater research effort has been directed toward alleviating the problem in sheep (Braden et al., 1964; Shutt, 1976).

The coumestrol content of whole alfalfa varies greatly from sample to sample (Lyman et al., 1959; Knuckles et al., 1976; Bickoff et al., 1969) and is influenced by environmental factors such as foliar disease and insect infestation (Loper and Hanson, 1964). Alfalfa estrogenic activity increases with the plant maturity, the number of cuttings, decreased sunshine, and increased moisture (Bickoff et al., 1969). For comparison with high alfalfa coumestrol values (11–118 ppm), the coumestrol content of 16 selected human food products of plant origin has been measured (Knuckles et al., 1976). Both fresh alfalfa sprouts (5 ppm) and fresh soybeam sprouts (71 ppm) were markedly higher in coumestrol than the other foods tested. Frozen green beans showed 1 ppm of coumestrol; other foods showed less than 1 ppm. The coumestrol content of alfalfa leaf protein concentrate, a promising protein source to fill future world food needs, can be controlled by the processing method used so that low-coumestrol (4–8 ppm) leaf protein concentrate can be prepared (Knuckles et al., 1976). Critical in this processing method is the expression of the protein-containing juice at pH 6, a pH that leaves more than 80% of the coumestrol present in the fibrous residue. Heat coagulation of the protein-containing juice at pH 8.5 yields leaf protein concentrate with a coumestrol content comparable to that of common vegetables.

A recent increase in the general public's awareness of good nutrition has led to the development of many "health foods" or dietary supplements. A common item in this category is alfalfa tablets, sold as a dietary supplement. The labels on bottles of these tablets do not indicate the processing procedure for their manufacture, so their coumestrol content is not known. This study reports the coumestrol concentrations found in three brands of commercial alfalfa tablets and one brand of alfalfa seed tablets.

EXPERIMENTAL SECTION

Chemicals and Reagents. Water was distilled and deionized. Solvents were high purity glass distilled. Coumestrol was purchased from Eastman Organic Chemicals and used without further purification. Alfalfa tablets were purchased from a local health food store and carried the following label information: sample 1, Alfalfa, Vitamin Supplements, Scottsdale, AZ 85254; sample 2, Alfalfa, Solgar Co., Inc., Lynbrook, NY 11563; sample 3, Nutrients Best Natural Alfalfa, Nutrients Best, Inc., Miami, FL 33166. One brand of alfalfa seed tablets, sample 4, KAL Alfalfa Seed, KAL, Inc., Canoga Park, California 91304, was also purchased locally.

Coumestrol Extraction. The extraction procedure of Knuckles et al. (1975) was followed with the omission of the initial grinding step. All samples were in tablet form and were readily crushed after soaking for a few minutes in water. Samples were stored in the dark at room temperature prior to HPLC analysis. Extractions were carried out in triplicate.

HPLC Analysis. Lookhart et al. (1978) gives detailed conditions necessary for the HPLC analysis of coumestrol. We altered these conditions slightly to increase the coumestrol retention time and to prevent overlapping of interfering peaks at the shorter coumestrol retention time.

Table I. Coumestrol Content of Commercial Alfalfa Tablets

sample source	ppm of coumestrol[a]
Vitamin Supplements	84.3 ± 8.4
Solgar Co., Inc.	193.6 ± 9.1
Nutrients Best	20.3 ± 0.30
KAL Alfalfa Seed	<0.1

[a] Reported as arithmetic mean ± standard deviation for six observations.

All HPLC analyses were performed with an Alltech modular unit employing a Milton Roy duplex pumping system, a 10-μL sample loop on a Valco valve, and a Laboratory Data Control fixed-wavelength UV detector (254 nm). An Alltech C_{18} 5-μm 25 cm × 4.6 mm column was used with a methanol and water (60:40 v/v) solvent system and a flow rate of about 1 mL/min (1700 psi). The water was adjusted to pH 3.5 with acetic acid. Under these conditions, the coumestrol retention time was about 18 min. Each of the triplicate samples was injected in duplicate; the arithmetic mean and standard deviation of each sample were reported.

Standard coumestrol solutions containing 0.12, 1.2, 4.8, and 12 ppm of coumestrol in absolute ethanol were prepared from a stock solution (120 ppm). Concentrations were calculated by use of a standard curve prepared from the peak areas of duplicate 10-μL injections of standards.

RESULTS AND DISCUSSION

The three sources of alfalfa tablets varied from 20 to 194 ppm in their coumestrol content (Table I). The one sample of alfalfa seed tablets analyzed showed no coumestrol at the detection limit of 0.12 ppm for 10-μL samples. The range of values obtained, 20–194 ppm, is not surprising when one considers that (1) the coumestrol content of alfalfa is known to vary widely depending on environmental factors and (2) the method of processing the alfalfa tablets is unknown. Knuckles et al. (1976) reported a range of 11–118 ppm of coumestrol in whole alfalfa while Lookhart (1980) found a range of 10–184 ppm. Bickoff et al. (1967) showed a 5-fold increase in coumestrol in plants infected with *Pseudopeziza medicaginis*, the common leaf spot organism, and also showed that the increase in coumestrol content was related to the alfalfa plant variety. Efforts have been made to eliminate, or at least limit, coumestrol content in products designed for human consumption (Knuckles et al., 1976; Lookhart, 1979). However, there is no indication of the processing procedure followed in the manufacture of alfalfa tablets for human consumption, and certainly, at least for the tablets from Solgar Co., Inc., no effort was made to minimize the coumestrol content (see Table I).

Nonsteroidal estrogens, such as coumestrol, may function in humans in the same manner as estradiol and may have profound effects on estrogen target cells (Martin et al., 1978; Verdeal et al., 1980). The inability of coumestrol to bind serum proteins (such as sex steroid-binding globulin or corticosteroid-binding globulin) serves to increase the concentration of free compound available at the target cell. Thus, coumestrol could be quite potent at relatively low blood concentrations (Martin et al., 1978; Shutt, 1976).

The label of the alfalfa tablets with highest coumestrol content (Solgar Co., Inc., 194 ppm) suggests the tablets should be used "as a dietary supplement" with the dosage of "three to 9 tablets daily as desired". The tablets weigh 0.67 ± 0.02 g each, so that the maximum label-recommended daily dosage would contain slightly more than 1.1 mg of coumestrol. However, many people are known to take twice this label-recommended dosage. These people

are thus adding more than 2 mg of coumestrol to their normal endogenous estrogen level in addition to any other exogenous estrogen sources such as birth control pills or estrogen replacement therapy. Certainly, this 1–2 mg/day is well below the 37 ppm in haylage found to elicit deleterious estrogenic effects when fed to cattle (Lookhart, 1980). Experiences with sheep and other animals have demonstrated wide species differences in susceptibility to phytoestrogens in feed (Shutt, 1976). Human susceptibility to phytoestrogens has been considered (Knuckles et al., 1976; Martin et al., 1978; Shutt, 1976) but not thoroughly investigated. Endogenous estrogen levels in humans vary greatly. Excessive alcohol consumption, age, liver function, nutrient deficiencies (Biskind, 1946), body weight and/or composition (Fishman et al., 1975), and diet (Hill et al., 1980) have all been shown to have profound effects on body estrogen levels and physical manifestations of these levels. The occurrence of coumestrol in human food products thus merits careful scrutiny.

Registry No. Coumestrol, 479-13-0.

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Stella D. Elakovich*[1]
Joan M. Hampton[2]

[1]Department of Chemistry
University of Southern Mississippi
Hattiesburg, Mississippi 39406–5043
[2]Hattiesburg, Mississippi 39401

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