Agronomic potential of sainfoin (Onobrychis viciaefolia) for Montana
by John L Holden

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
Montana State University

Abstract:
A study was initiated to evaluate the potential of sainfoin for production in Montana.

A search of the literature revealed sainfoin to be a deep-rooted, long-lived, tetrapoid, perennial legume particularly adapted to dry, well-drained calcareous soils. It is reported not to cause bloat and outyield-ed all other legumes at the Montana Experiment Station when harvested for only one cutting of hay.

Research was undertaken to evaluate the seedling emergence as affected by seeding depth, the protein quality of the seed, and the comparative seasonal chemical composition of sainfoin to other legumes including estrogenic activity.

The experiment on seeding depth indicated that sainfoin will allow a higher percentage of seedling emergence than alfalfa from a depth of three inches or less.

The protein quality of sainfoin seed is similar to that of soybean oil meal and warrants consideration as a source of supplemental protein for livestock feeding.

The seasonal protein content of sainfoin was found to decrease with advancing maturity and dry matter percentage increases. A high negative correlation (-.89) was calculated for protein as related to dry matter percentage in legumes which could lead to a quick, comparative method of calculating crude protein content of legume forages. Sainfoin matures at about the same rate as alfalfa. It has a higher leaf to stem ratio than alfalfa and is lower in crude fiber and protein. Sainfoin has a high percentage of nitrogen free extract in comparison to other legumes which, coupled to its lower protein content, makes it worthy of consideration as a silage crop.

All three stages of red clover and the regrowth stage of alfalfa produced a significantly higher estrogenic response than any of the other legumes. Sainfoin did not exhibit a measurable amount of estrogenic activity at any stage of growth.

Sainfoin possesses potential for both forage and send production in Montana, especially in dryland areas where one cutting of hay is harvested, or on irrigated land where a high-yielding silage crop is desired.

AGRONOMIC POTENTIAL OF SAINFOIN (ONOBRYCHIS VICIAEFOLIA) FOR MONTANA

by

JOHN L. HOLDEN

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

Agronomy

Approved:

Head, Major Department

Chairman, Examining Committee

Dean, Graduate Division

MONTANA STATE COLLEGE
Bozeman, Montana

May, 1963

ACKNOWLEDGMENT

The author wishes to acknowledge the counsel and advice of Professor Robert F. Eslick, under whose guidance this study was conducted. Appreciation is expressed to Assistant Professor Arthur S. Hoversland of the Animal Science Department for his help and encouragement during the bioassay study and to Dr. Oscar O. Thomas, Dr. Erhardt R. Hehn, and Dr. Lark P. Carter for serving as members of the graduate committee.

Special acknowledgment is due Dr. Richard R. Roehm and Mrs. Helen L. Mayfield of Home Economics Research for their generous cooperation and use of their small animal laboratory facilities.

The author also wishes to thank Professor Fred S. Willson, head of the Animal Science Department, for use of the facilities in the Animal Nutrition Laboratory.

TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGMENT	iii
LIST OF TABLES	v
LIST OF FIGURES	vii
ABSTRACT	viii
INTRODUCTION	1
LITERATURE REVIEW	3
SEEDING DEPTH	12
Materials and Methods	12
Results and Discussion	12
PROTEIN QUALITY OF SAINFOIN SEED	15
Materials and Methods	15
Results and Discussion	16
SEASONAL CHEMICAL COMPOSITION	19
Materials and Methods	19
Results and Discussion	20
ESTROGENIC ACTIVITY	29
Materials and Methods	29
Results and Discussion	34
SUMMARY	47
LITERATURE CITED	49

LIST OF TABLES

		Page
Table I.	Percentage of viable sainfoin and alfalfa seeds which germinated and emerged from various seeding depths	13
Table II.	Composition of rations used to evaluate protein quality of sainfoin seed meal	15
Table III.	Average rat weight gains and feed efficiency during a feed trial conducted using pigweed seed meal, sainfoin seed meal and soybean oil meal as protein sources	16
Table IV.	Growth stages of 8 legumes and orchardgrass on various harvest dates at Bozeman in 1962	21
Table V.	Height in inches of 8 legumes and orchardgrass on various harvest dates at Bozeman in 1962	22
Table VI.	Dry matter content of 8 legumes and orchardgrass on various harvest dates at Bozeman in 1962	23
Table VII.	Crude protein content of 8 legumes and orchardgrass on various harvest dates at Bozeman in 1962	24
Table VIII.	Leaf-stem comparison of 7 legumes and orchardgrass at hay stage at Bozeman in 1962	26
Table IX.	Nutrient composition of leaf and stem separations of alfalfa and sainfoin hays grown at Bozeman in 1962	27
Table X.	Estrogenic activity of various levels of diethylstilbestrol added to a control ration	37
Table XI.	Estrogenic activity of three stages of alfalfa with different storage procedures and sainfoin seed, when fed without extraction as part of the mouse ration	38

Page

Table XII. Estrogenic activity of increasing levels of extract from vegetative stages of alfalfa and red clover using Bickoff Fresh Forage Extraction Method.......... 40

Table XIII. Estrogenic activity of three stages of alfalfa during two trials using the Canadian Extraction Method.......... 42

Table XIV. Estrogenic activity of three stages of legumes and sainfoin seed using the Canadian Extraction Method.......... 43

Table XV. Estrogenic activity comparison of Bickoff Fresh Forage Extraction Method with Canadian Dry Forage Extraction Method on hay stage forage of various legumes.......... 44

Table XVI. Estimated diethylstilbestrol equivalency of red clover and alfalfa in micrograms per pound of dry matter.......... 46

LIST OF FIGURES

Page

Figure 1. Diethylstilbestrol dose response curve and regression of uterine weight as percentage of body weight on micrograms of diethylstilbestrol in feed at levels of 0 to .06 micrograms per gram of feed.............. 36

ABSTRACT

A study was initiated to evaluate the potential of sainfoin for production in Montana.

A search of the literature revealed sainfoin to be a deep-rooted, long-lived, tetrapoid, perennial legume particularly adapted to dry, well-drained calcareous soils. It is reported not to cause bloat and outyielded all other legumes at the Montana Experiment Station when harvested for only one cutting of hay.

Research was undertaken to evaluate the seedling emergence as affected by seeding depth, the protein quality of the seed, and the comparative seasonal chemical composition of sainfoin to other legumes including estrogenic activity.

The experiment on seeding depth indicated that sainfoin will allow a higher percentage of seedling emergence than alfalfa from a depth of three inches or less.

The protein quality of sainfoin seed is similar to that of soybean oil meal and warrants consideration as a source of supplemental protein for livestock feeding.

The seasonal protein content of sainfoin was found to decrease with advancing maturity and dry matter percentage increases. A high negative correlation (-.89) was calculated for protein as related to dry matter percentage in legumes which could lead to a quick, comparative method of calculating crude protein content of legume forages. Sainfoin matures at about the same rate as alfalfa. It has a higher leaf to stem ratio than alfalfa and is lower in crude fiber and protein. Sainfoin has a high percentage of nitrogen free extract in comparison to other legumes which, coupled to its lower protein content, makes it worthy of consideration as a silage crop.

All three stages of red clover and the regrowth stage of alfalfa produced a significantly higher estrogenic response than any of the other legumes. Sainfoin did not exhibit a measurable amount of estrogenic activity at any stage of growth.

Sainfoin possesses potential for both forage and seed production in Montana, especially in dryland areas where one cutting of hay is harvested, or on irrigated land where a high-yielding silage crop is desired.

INTRODUCTION

Dr. R. G. Stapledon, famous British grass specialist, has said, "No grassland is worthy of the name and hardly worth bothering with unless a legume is at work. Find or breed the right legume for every corner of the world and you have developed good grassland in every corner of the world."

The "right" legume for Montana would combine the characteristics of high nutrient production, non-bloating, palatability, competitive ability, seedling vigor, ample seed production, long life, disease and drought resistance, winter hardiness, and adaptability to our soils. Our present legumes have some of these attributes but not all. Birdsfoot trefoil is long-lived, semi-winterhardy, sometimes high yielding, somewhat drought resistant, alkali tolerant and has been reported not to cause bloat. However, it is very slow to establish, may be somewhat lacking in palatability, and will not withstand grazing under drought conditions. Red clover and alsike clover are short-lived and not drought resistant. White clover is low yielding and its close relative, Ladino, lacks winterhardiness, and has high moisture and fertility requirements. The sweetclovers are biennial, susceptible to the sweetclover weevil and contain the undesirable compound coumarin. Alfalfa is good from almost all standpoints except that it may represent a serious bloat hazard in pastures.

Sainfoin (*Onobrychis viciaefolia*) is a deep-rooted, long-lived, tetrapoid, perennial legume introduced from Europe which comes close to possessing all the desired characteristics of a legume for Montana. It is particularly adapted to dry calcareous soils. Piper and Mansfield state, "that sainfoin has never been known to cause bloat." Its common name sainfoin, French for "healthy hay", and the Latin species name Onobrychis, meaning "that for which asses bray", may aptly describe its forage qualities.

These factors were thought to make sainfoin worthy of more detailed evaluation as a possible legume for production in Montana.

LITERATURE REVIEW

The sainfoin plant consists of 3 to 32 stout, erect stems which arise from a branched crown (40). "The leaves are odd-pinnate with 13 to 15 leaflets. The rose colored (rarely white) flowers are in an erect, close raceme 2 to 5 inches long. The roots are reported to reach a diameter of 2 inches and extend to a depth of 20 feet or more. The culture of sainfoin probably dates back about 400 years. It was first cultivated in France; the first definite record according to Vianne was in 1582." (34) At present sainfoin is commonly grown in parts of England and France. Sainfoin (<u>Onobrychis altissima</u>) comes from the Near Eastern Center of Origin. Several species occur wild in that area and in the southern half of Europe and extend eastward toward Russia where extensive research is being done on sainfoin for forage (25), (34).

In England, information obtained from production of sainfoin hay, which is highly prized by racing stables for feeding brood mares and colts, has led to its use on much dry calcareous land, which previously had been nearly valueless (34).

Numerous popular articles have been published in the United States on sainfoin's value as a bee forage (1), (11), (24), (25), (31) and potential for livestock utilization (3) but very little sainfoin is being grown at present.

The two varieties of sainfoin grown in England, common and giant, may have possibilities for production in Montana. They are particularly well adapted to dry, well-drained calcareous soils with a low water table

(26). Pellet, (31), reported them extremely drought and cold resistant. Common differs from giant sainfoin in having finer stems, a more prostrate growth habit, greater persistency of stand, and inability to flower twice a season (18). The first cutting of giant is usually taken for hay and the second left for seed production (3). Giant is usually seeded as a short rotation crop, 2 to 3 years, and common as a long rotation crop, 4 to 7 years. After 7 or 8 years of sainfoin production, good soils become sick in a manner annalogous to "clover sick" soils and must be reseeded to another crop; however, this doesn't occur on the dryer, less fertile soils (34). Eslick (15) reported a stand at Hall, Montana, which has been in existence since 1898. Both giant and common sainfoin are reported to be similar to alfalfa in their ability to withstand frequent close grazing or clipping (16).

Sainfoin is adaptable to good irrigated soils (34). Research by Cooper (10) at the Montana Agricultural Experiment Station showed that sainfoin outyielded all other legume species when compared with first cutting hay yields.

The nutritive content of a plant, within limits, is the final determining factor in its value as a forage crop. Norton (29) states, "There are two factors which greatly influence the feeding value of alfalfa hay; namely, the proportion of leaves to stems and the crude protein content." Morrison's Feeds and Feeding (27) doesn't list the chemical composition of sainfoin. Baker (4), in England, found that like most forages, sainfoin has a lower crude protein content, and a higher crude fiber content as the plant advances toward maturity. The mineral content was found to be somewhat variable. The ratio of leaf dry matter to total dry matter decreases with maturity of the plant (4). The leaf composition is fairly constant irrespective of growth stage, number of cuttings and season; with leaf fiber content being remarkably constant in contrast to that of stem, where it increases with maturity of the plant. The leaf contains more crude protein, ether extract, and mineral matter, particularly calcium, than the stem. (4) Changes which occur in the composition of sainfoin are postulated to be due to variation in stem composition and leaf-stem ratio. Sainfoin is not considered to be a particularly rich source of beta-carotene (4). Baker (4) lists the following as the average composition of 35 samples of sainfoin hay grown in England. For comparison, data on alfalfa from Morrison (27) is given.

Composition of Dry Matter

Constituent	Sainfoin %	Alfalfa %
Moisture	0.0	9.5
Crude Protein	15.0	15.3
Ether Extract	1.8	1.9
Crude Fiber	30.8	28.6
N. Free Extract	45.5	36.7
Total Ash	6.9	8.0
Silica	0.8	-----
Calcium	1.84	1.47
Phosphorous	0.63	0.24
Potassium	1.52	1.97
Chlorides	0.53	-----

Norton (29) reports 40.4 as the average leaf percentage of first cutting alfalfa hay in comparison to 32.3 as the average leaf percentage of sainfoin hay given by Baker (4).

In recent years the estrogenic activity of forages has received consideration in evaluating forages.

The term "estrogen" can be defined as any compound or substance which is capable of causing sexual development in the female, including changes in the vaginal epithelium, hypertrophy of the uterus and mammary glands, and the development of female secondary sex characteristics (44). Estrogenic substances were first discovered in plants in 1926 by Loewe (38). Since that time approximately 50 species of plants have been shown to possess varying degrees of estrogenic activity (32). Interest in forage estrogens was renewed in 1941 when widespread breeding disorders of sheep in Western Australia were found to be caused by the high estrogenic content of subterranean clover (Trifolium subterranean). Since that time, other forage plants which have been found to possess estrogenic compounds of varying activity are red clover (Trifolium pratense), alfalfa (Medicago sativa), white clover (Trifolium repens), strawberry clover (Trifolium frageferum), Ladino clover (Trifolium repens, latum), and birdsfoot trefoil (Lotus corniculatus) (38). Soybean oil meal is also reported to contain estrogenic compounds (38). Red clover is most consistently reported as having estrogenic activity at all stages of growth. It has caused breeding disorders in sheep in Ohio and Oregon (9). Alfalfa varieties are reported to have wide differences in estrogen content (32). Youngman (44) reports Ladak alfalfa in Washington to have increasing estrogenic activity with maturity. Hay samples of alfalfa, alsike clover, white clover and red clover have been reported to possess estrogenic activity (38). Alfalfa silage has been found to possess significantly greater estrogenic activity than fresh alfalfa or alfalfa hay (32), (44). White clover, birdsfoot trefoil, and alfalfa have been reported to be highest in estrogen content during early spring growth (38). The estrogenic activity of forages seems to vary with variety, stage of growth, number of cuttings and environment (32), (44).

Various conflicting theories exist as to the site of production of estrogens in plants. Estrogens occur earliest in the chloroplast fraction, so this would seem the most likely site of production (23).

The estrogenic compounds which have been isolated and identified are the flavinoids, genistein, its glycoside genisten, diadzein, its glycoside diadzin, formononetin, biochanin A and a coumarin-like compound, coumestrol. These all have similar chemical structures which differ mainly in number and location of hydroxyl and methoxyl groups (38). Coumestrol, which has been isolated in all legumes tested, possesses a higher estrogenic activity than any of the other compounds (9).

Quantitative and qualitative determination of estrogens in plant and animal material can be accomplished by chemical methods and bio-assay procedures. Chemical procedures are time consuming, especially with plant materials, since chromatrography and accurate analytical methods are necessary. When the chemical and physical properties of estrogens are not desired, a gross quantitative estimate of the estrogenic activity can be obtained by use of bio-assay techniques (38). With forages, this involves extraction of the estrogen with a solvent and then adding the extract to a control ration which is fed to immature female mice or rats. There is no difference in reliability of results, but mice are less expensive. The animals are fed from three to ten days and then sacrificed. The resultant uterine weight increase is used as a measure of estrogenic activity. The results are usually stated as equivalent to the amount of diethylstilbestrol which would cause the same uterine response (38). Bickoff *et al.* (8) found that acetone, alcohol, or a 2:1 benzene-alcohol mixture are equally effective in extracting estrogenic compounds from fresh or dried Ladino clover. Drying a forage can cause up to a 75% loss in estrogenic activity. Estrogenic compounds are fairly stable after extraction (9).

In addition to affecting reproduction, plant estrogens have been observed to affect the composition and production of milk and to stimulate growth in castrated male animals (9). Bickoff (9) states, "It now appears that we may need two separate types of forages. One of these would be a low-estrogen forage for breeding stock, because the naturally-occurring estrogens are not desirable here. The second type would be a high-estrogen forage for fattening steers, wethers and poultry." Data has not been published concerning the estrogenic activity of sainfoin or sainfoin seed.

Efficient production of legume forages is dependent upon successful stand establishment. Among factors influencing establishment are seed size, seed viability, rate of seeding, depth of seeding and variety.

Sainfoin seed is produced by the plant in one-seeded pods which are described by Piper (34) as being "brown, indehiscent, lenticular, and reticulated on the surface." Actually, the pod is bean-shaped and bilaterally compressed. It has a rough net veined appearance, and is sometimes referred to as a "cockshead" when it has spines protruding from the ventral edge or keel. Evidence indicates that the spined characteristic is controlled by one pair of genes with spiny dominant to spineless (40).

Sainfoin grown for seed production may produce 25 to 30 bushels of seed in the pod per acre (3). Like alfalfa, it is entomophalous, requiring insect cross-pollination for maximum seed set (15). The podded seed weighs about 30 pounds per bushel, of which one-third, by weight, is pod and shriveled seeds (40). Sainfoin seed remains viable under high drying temperatures after harvest. It is safe to store at 12% moisture and is reported in England to lose its viability after three years storage (30). Sainfoin seed is sold commercially in two forms, milled and unmilled. Milled seed has the pod removed and is kidney-shaped, varying from yellowish green to dark brown in color. Sainfoin seed in the pod weighs 23 grams per 1000 and milled seed weighs 15 grams per 1000 (39). In comparison alfalfa seed weighs 2 grams per 1000 (37). Germination is more uniform from milled seed due to reduction of hard seeds during milling and removal of the seed pod which has been proved to provide mechanical resistance to radicle emergence (40). Unmilled seed retains its viability for a longer period (39).

The recommended seeding rate for alfalfa and sainfoin in England is 22 and 40 pounds of milled seed per acre, respectively (30). In Montana the recommended seeding rate for alfalfa is 10 to 12 pounds per acre.

Piper (34) states that sainfoin should be seeded at a depth of one-half inch or more. The most satisfactory seeding depth for small seeded legumes and grasses in this country is 0.5 inch (5). Two inches is the recommended seeding depth for soybeans(28). Erickson (13) found that as depth of seeding increased, the advantage of large seed over small seed in stand establishment of alfalfa became increasingly apparent. This would cause one to wonder just what the optimum seeding depth for sainfoin might be. Sainfoin seed requires inoculation for proper nitrogen fixation and growth on soils which had not previously grown sainfoin (1). Piper (34) recommends spring seeding, since fall seedings are more apt to winterkill.

Woodman (42) gives the chemical composition of sainfoin seed as follows:

	Unmilled seed	Milled seed
	%	%
Moisture	12.01	8.98
Crude Protein	26.38	36.63
Ether Extract	5.96	7.28
N. Free Extract	33.74	34.06
Crude Fiber	17.86	9.46
Ash	4.05	3.58
Calcium	1.02	.24
Phosphorous	.91	1.15
Chlorides	.11	.11

For a long time protein has been the most expensive ingredient and a limiting factor of livestock rations. Soybean oil meal is commonly used as a protein supplement in livestock rations, including dog and cat foods, because of its high protein content and essential amino acid balance in relation to cereal grains. Recent research indicates that the limiting amino acid for soybean oil meal is methionine, but even without the addition of methionine, it is one of the best quality plant proteins available for livestock feeding (2). Soybean oil meal is listed by Morrison (27) as being about 44 % crude protein. Milled sainfoin seed compares favorably to soybean oil meal in protein percentage, and might be considered for commercial production if it was equal to soybean oil meal in amino acid balance. Soybean oil meal sells commercially at the present time for $83 a ton. Woodman (42) indicated that lambs did very well on unmilled sainfoin seed as a protein supplement and would have equaled gains of lambs on lucerne and clover seed if the pod had been removed.

The search of literature on sainfoin indicated that information was lacking in the areas of recommended seeding depth, protein quality of the seed, and comparative seasonal chemical composition including estrogenic activity. Research was undertaken to evaluate these qualities of sainfoin.

SEEDING DEPTH

Materials and Methods

Because of the exceptionally large size of sainfoin seed and lack of information regarding proper seeding depth, an experiment was designed to evaluate the effect of seeding depth on the resulting seedling emergence of sainfoin.

One hundred alfalfa seeds and an equal number of sainfoin seeds were planted at eight different depths, starting at one-half inch and increasing in one-half inch increments to four inches, in soil on a bench in the greenhouse. A split-plot experimental design was used with seeding depths assigned at random to the main plots and species to subplots. Three replications were planted. Germination percentage of the seed was obtained before planting. Relative rate of emergence was noted and results were compiled as percentage of viable seed emerging. Alfalfa was included in the experiment as a check.

Results and Discussion

The germination percentage of sainfoin seed and alfalfa seed was 78 and 95 respectively. The sainfoin seed had been stored for three years which refutes data by Owen (30) that indicated sainfoin seed loses its viability during that period of time.

The alfalfa seedlings emerged a day earlier than the sainfoin seedlings at depths of seeding to two inches. Sainfoin at greater depths had the faster emergence. The sainfoin seedlings emerged with the hypocotyl pushing the cotyledons straight up through the soil as did alfalfa. This

Table I. Percentage of viable sainfoin and alfalfa seeds which germinated and emerged from various seeding depths. 1/

Seeding depth in inches	Percent age of sainfoin seeds emerging	Percent age of alfalfa seeds emerging
.5	88.5 a b	60.2 a
1.0	97.0 a	57.9 a
1.5	76.9 a b	56.3 a
2.0	88.9 a b	37.2 a b
2.5	63.7 b c	22.8 b c
3.0	38.9 c	17.6 b c
3.5	9.4 d	1.1 c
4.0	1.3 d	1.4 c

1/ Values underlined within a seeding depth are significantly different at P = .05.
Values within the legume followed by the same letter are not significantly different at P = .05 (12).

is different from some legumes, such as beans, in which the hypocotyl is sharply bent and pulls the cotyledons to the soil surface.

The results of the experiment are shown in Table I as percentage of viable seeds which emerged from the soil as seedlings.

Alfalfa had an emergence of 60.2 % from the 0.5 inch seeding depth which is comparable to field emergence. Sainfoin exhibited good emergence from the first four seeding depths, fair emergence in the 2.5 and 3 inch seeding depths and poor in the 3.5 and 4 inch depths. In comparison, alfalfa had good emergence in the first three seeding depths, fair emergence in the 2, 2.5 and 3 inch seeding depths and poor in the 3.5 and 4 inch depths.

The experiment was designed to place maximum precision in statistical analysis on the seeding depth by legume interaction. A significant difference greater than P = .05 existed between legumes. Seeding depth differences were significant at P = .01 with the interaction having significance greater than P = .05.

This experiment indicates that sainfoin is superior to alfalfa in seedling emergence and will produce a greater percentage of seedlings than alfalfa from a seeding depth of 3 inches or less.

PROTEIN QUALITY OF SAINFOIN SEED

Materials and Methods

The experiment herein described was designed to compare the protein quality (essential amino acid balance) of sainfoin seed (Onobrychis viciaefolia) and pigweed seed (Amaranthus retroflexus) with that of soybean oil meal (Glycine max) and to measure its effect on rat growth and feed consumption.

Fifteen three-week-old female white rats were divided into three equal weight groups of five rats each.

Rations were formulated to contain 10% crude protein, on the basis of work by Rama Rao et al. (35) which indicated that the minimum rat requirement of protein for maximum nitrogen retention is 10% and the minimum requirement for maximum growth is 8.8%. Crude protein contents of unhulled sainfoin seed, soybean oil meal and pigweed seed used in the rations were 24.6%, 42.0% and 15.0% respectively.

Table II shows the rations used for the experiment.

Table II. Composition of rations used to evaluate protein quality of sainfoin seed meal.

Ingredients	Pigweed seed meal ration %	Sainfoin seed meal ration %	Soybean oil meal ration %
Vitamin premix	1.0	1.0	1.0
U.S.P. 14 salt mix	4.0	4.0	4.0
Alpha cellulose	5.0	5.0	5.0
Corn oil	10.0	10.0	10.0
Corn Starch	13.3	39.3	56.2
Protein source	67.7	40.7	23.8

Each group of rats was fed ad libitum for 28 days. Weights were taken at about 10 day intervals during the feeding trial and feed consumption was noted.

Results and Discussion

The soybean oil meal ration and sainfoin seed meal ration produced significantly (P = .05) greater rat gains than the pigweed seed meal ration during the last 17 days of the feeding trial (Table III). There was no significant difference between weight gains of rats fed the sainfoin and pigweed rations during the first 11 days of the experiment, nor between weight gains from the sainfoin and soybean rations throughout the trial.

Table III. Average rat weight gains and feed efficiency during a feed trial conducted using pigweed seed meal, sainfoin seed meal and soybean oil meal as protein sources.1/

Treatment	Weights taken in grams Int. wt.	11 day gain	11-21 day gain	21-28 day gain	Final wt.	Total gain	Avg. daily	Grs. feed per 100 grs. gain
Soybean oil meal ration	56.2	38.6a	28.1a	14.2a	136.7	80.5a	2.88a	396
Sainfoin seed meal ration	55.9	31.5ab	30.4a	10.5a	128.3	72.4a	2.59a	460
Pigweed seed meal ration	57.1	20.3b	11.0b	3.3b	91.7	34.6b	1.24b	643

1/ Differences among rations are significant at P = .05 if not followed by the same letter (12).

Kumpta and Harper (22) stated that, "A fall in food intake can usually be detected within a short time after an animal has been fed ad libitum a diet in which an amino acid imbalance has been created." This occurred with the rats on the pigweed seed ration. They ate a total of 1112 grams of feed compared to 1595 grams of feed for the rats on soybean and 1664 grams of feed for the rats on sainfoin. They were also more nervous and irritable than the rats in the other two groups. This agrees with previous research by Young and Dunn (43).

The higher feed consumption and lower feed efficiency of the rats on sainfoin as compared to those on soybean may have been due to the pods on the sainfoin seed which were ground with the seed and mixed as part of the ration. These pods were unpalatable and the rats wasted part of them. The pods are high in fiber (42) and should contain less energy than an equal amount in weight of corn starch which was their counterpart in the soybean oil meal ration.

The author surmises, as did Woodman and Evans (42), that milled sainfoin seed with the pods removed would compare much more favorably to soybean oil meal with respect to protein quality.

In England, sainfoin is reported to produce about 600 pounds of milled seed per acre (3). Compared to the present price of soybean oil meal, this would be worth about $24 per acre. Sainfoin seed could be harvested every year. The value of two years' production of sainfoin seed would be equal to a 24 bushel wheat crop in an alternate year - summer fallow cropping system.

The value of the seed as a protein supplement for the livestock industry plus the forage production of the plant makes sainfoin worthy of consideration for production in Montana.

SEASONAL CHEMICAL COMPOSITION

Materials and Methods

In order to obtain a comparison of sainfoin with other forage legumes, weekly harvests were made from eight legumes and one grass grown in plots located approximately five miles west of Bozeman on the Montana Agricultural Experiment Station, Crops and Soils Field Research Laboratory. The forages compared were sainfoin (*Onobrychis viciaefolia*), Ladak alfalfa (*Medicago sativa*), tetrapoid alsike clover (*Trifolium hybridium*), Kenland red clover (*Trifolium pratense L.*), Ladino clover (*Trifolium repens*), Tana birdsfoot trefoil (*Lotus corniculatus*), cicer milkvetch (*Astragulus cicer*), sickle milkvetch (*Astragulus falcatus*) and orchardgrass (*Dactylis glomerata*).

The harvests were made on Friday afternoon each week. The height of cutting was 2 inches. Height and stage of maturity were noted at time of harvest. The samples were selected at random from the plots, placed in paper bags and oven dried in the forage dryer at about 68^{o} C for three days. Leaf and stem separations were made at the hay stage. Ladino clover was not included in the comparison until August 3, when samples were taken on regrowth from the hay cutting. Dry matter percentage was determined and samples ground through a 1/32 inch screen in a Wiley mill for crude protein determination by the Kjeldahl method. Samples of leaf and stem separation from alfalfa and sainfoin were analyzed by the Chemistry Research Laboratory for crude fiber, ether extract, and total ash. Nitrogen free extract was calculated using the other values.

Results and Discussion

The relative rate of maturity of sainfoin in relation to other legumes and orchardgrass is shown in Table IV. Sainfoin reached the hay stage one day earlier than alfalfa. It did not exhibit leaf loss in comparison to alfalfa which, at Bozeman, normally loses some of its lower leaves before reaching the hay stage. Sainfoin and the two milkvetches were less affected by frost in the fall than the other legumes. Sainfoin was the tallest of all legumes during the development of the first cutting, Table V.

Comparative seasonal variation in dry matter is shown in Table VI. Variations from normal for some dates may be attributed to rain and moisture on the forage when the samples were harvested. Sainfoin did not exhibit as high a dry matter percentage as might be expected considering its height and large, rank growing stems.

The protein content of sickle milkvetch was considerably higher than the other legumes throughout the experiment, Table VII. Sainfoin and alfalfa had a lower protein content than previously reported (4), (27). This could have been due to environmental, variety, or inoculation effects. The trefoil plants were yellow and appeared to be suffering from improper inoculation or a lack of nitrogen. This could account for its rapid decline in protein content.

The protein and dry matter comparisons were not statistically analyzed because just one sample was taken in the field to obtain the data of

Table IV. Growth stages of 8 legumes and orchardgrass on various harvest dates at Bozeman in 1962.1/

Date of Harvest	Alfalfa	Alsike Clover	Birdsfoot Trefoil	Cicer Milk-vetch	Red Clover	Sickle Milk-vetch	Sainfoin	Ladino Clover	Orchard-grass
				First Crop Growth					
5-18	veg. *	veg.	veg.	veg.	veg.	veg.	veg.	---	veg.
5-25	veg.	veg.	veg.	veg.	veg.	veg.	veg.	---	veg.
6-1	veg.	veg.	1st buds	veg.	veg.	veg.	veg.	---	boot
6-8	veg.	veg.	1st bloom	veg.	veg.	1st buds	veg.	---	heading
6-15	1st leaf loss	1st bud	30% bloom	veg.	1st bloom	adv. bud	1st buds	---	heading
6-20	---	---	75% bloom	---	---	---	---	---	pre-bloom
6-22	1st buds	1st bloom	Fullbloom	1st bud	20% bloom	pre-bloom	1st bloom	---	blooming
6-26	---	---	---	---	---	---	50% bloom	---	---
6-27	5% bloom	---	---	---	---	pods form	---	---	blooming
6-29	35% bloom	75% bloom	75% pods	adv.bud	fullbloom	50% pods	75% bloom	---	blooming
7-2	---	fullbloom	---	---	---	---	---	fullbloom	---
7-5	---	---	---	50% bloom	---	---	---	---	---
7-6	65% bloom	15% d.f.*	85% pods	60% bloom	40% d.f.	100% pods	75% d.f.	---	s.f.*
7-14	5% pods	50% d.f.	100% pods	15% pods	80% d.f.	s.f.	90% pods	---	s.f.
7-20	25% pods	75% d.f.	s.f.	75% pods	90% d.f.	s.f.	s.f.	---	s.f.
7-27	50% pods	95% d.f.	s.f.	100% pods	s.f.	s.f.	s.f.	---	s.f.
				Regrowth After Cutting					
8-3	veg.	veg.	25% bloom	veg.	5% bloom	veg.	veg.	10% bloom	veg.
8-10	veg.	5% bloom	40% pods	veg.	15% bloom	veg.	veg.	20% bloom	veg.
8-17	15% bloom	10% bloom	90% pods	veg.	50% bloom	veg.	10% bloom	35% bloom	veg.
8-24	40% bloom	20% bloom	100% pods	veg.	70% bloom	veg.	20% bloom	50% bloom	veg.
8-31	50% bloom	15% d.f.	s.f.	veg.	30% d.f.	veg.	35% bloom	100% bloom	veg.
9-7	50% d.f.	75% d.f.	s.f.	veg.	60% d.f.	veg.	60% d.f.	45% d.f.	veg.
9-14	65% d.f.	95% d.f.	s.f.	veg.	85% d.f.	veg.	90% d.f.	90% d.f.	veg.
9-21	frosted	s.f.	s.f.	veg.	95% d.f.	veg	p.f.*	90% d.f.	veg.

1/ Underscored stages indicate dates and stages considered to be correct for haying; samples for leaf-stem separations were taken on these dates.

* veg. = vegetative stage. d.f. = dried flowers after blooming. s.f. = seeds forming. p.f. = pods forming.

Table V. Height in inches of 8 legumes and orchardgrass on various harvest dates at Bozeman in 1962.1/

Date of Harvest	Alfalfa	Alsike Clover	Birdsfoot Trefoil	Cicer Milkvetch	Red Clover	Sickle Milkvetch	Sainfoin	Ladino Clover	Orchard-grass
				First Crop Growth					
	in.	in.	in.	in.	in.	in.	in.	in.	in.
5-8	12	4	5	5	8	6	8	-	12
5-25	16	9	9	7	9	10	12	-	14
6-1	19	12	12	11	15	15	16	-	19
6-8	22	14	14	15	19	20	20	-	24
6-15	25	20	17	16	22	21	23	-	30
6-20	-	-	20	-	-	-	-	-	-
6-22	29	24	21	21	24	27	33	-	44
6-26	-	-	-	-	-	-	34	-	-
6-27	32	-	-	-	-	29	-	-	47
6-29	32	24	22	22	24	30	36	-	48
7-2	-	24	-	-	-	-	-	14	-
7-5	-	-	-	24	-	-	-	-	-
7-6	34	24	24	25	29	33	38	-	48
7-14	36	24	24	26	29	33	38	-	48
7-20	36	24	24	26	29	33	38	-	48
7-27	36	24	24	26	29	33	38	-	48
				Regrowth after cutting					
8-3	14	7	13	2	16	5	9	7	7
8-10	16	9	13	4	20	7	12	10	12
8-17	21	11	13	6	24	9	17	10	13
8-24	22	10	14	8	24	10	18	12	14
8-31	21	10	14	6	24	10	22	11	13
9-7	18	10	13	8	24	10	20	10	13
9-14	18	9	13	8	23	9	20	10	13
9-21	16	9	13	9	23	11	19	9	12

1/ Values underlined are heights at hay stage.

Table VI. Dry-matter content of 8 legumes and orchardgrass on various harvest dates at Bozeman in 1962.[1]

Date of Harvest	Alfalfa	Alsike Clover	Birdsfoot Trefoil	Cicer Milkvetch	Red Clover	Sickle Milkvetch	Sainfoin	Ladino Clover	Orchard-grass
	%	%	%	%	%	%	%	%	%
					First crop growth				
5-18	16.8	17.1	15.5	10.1	18.0	23.7	16.0	----	21.6
5-25	14.8	15.0	14.3	14.4	15.8	17.8	14.3	----	14.7
6-1	15.9	12.9	14.4	13.7	13.7	15.9	14.5	----	19.2
6-8	17.0	13.3	16.1	14.2	14.4	17.6	15.3	----	19.5
6-15	18.9	11.9	16.3	13.7	18.2	18.5	16.4	----	24.3
6-20	----	----	18.5	----	----	----	----	----	----
6-22	23.9	15.5	19.1	16.2	22.9	23.7	20.9	----	29.5
6-26	----	----	----	----	----	----	27.3	----	----
6-27	28.4	----	----	----	----	33.2	----	----	39.1
6-29	31.2	18.2	21.8	20.9	27.2	23.0	29.0	----	36.0
7-2	----	21.9	----	----	----	----	----	23.6	----
7-5	----	----	----	23.8	----	----	----	----	----
7-6	33.2	22.0	23.3	24.9	28.1	26.6	34.4	----	48.2
7-14	31.5	22.5	23.2	27.3	27.3	30.0	37.5	----	38.9
7-20	31.9	24.5	23.6	27.4	30.5	31.3	33.3	----	33.7
7-27	30.3	24.3	25.4	26.3	30.5	42.3	34.7	----	49.1
					Regrowth after cutting				
8-3	21.1	16.0	24.6	22.8	19.3	27.0	20.9	17.4	27.4
8-10	24.4	17.7	25.0	17.2	19.7	23.9	20.0	20.6	24.8
8-17	24.8	18.4	26.3	14.9	21.3	23.0	20.9	16.6	28.9
8-24	29.6	22.7	29.0	22.0	25.5	28.0	26.2	19.5	33.0
8-31	29.3	21.9	23.8	18.3	27.0	25.0	24.0	17.5	29.0
9-7	35.5	27.5	28.3	26.3	27.9	27.3	26.5	24.0	30.9
9-14	38.0	26.7	28.5	24.0	28.4	27.5	29.4	25.9	35.1
9-21	45.0	32.5	29.3	26.6	35.6	31.2	31.2	24.7	32.2

1/ Values underlined are dry matter percentage at hay stage.