Yield and yield components of sainfoin (Onobrychis viciaefolia Scop.) seed and an evaluation of its use as a protein supplement

by Raymond Lee Ditterline

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Crop and Soil Science
Montana State University

Abstract:
Yield and yield components of sainfoin seed, and protein quality of sainfoin seed were studied in separate experiments. The first experiment was a study of the components of seed yield in sainfoin and their heritabilities. Components studied included stems per plant, racemes per plant, racemes per stem, florets per raceme, seed per raceme, percent seed set, weight per 100 seeds, and seed yield. Crude protein content of the seed, plant height and lodging were also studied. Significant differences between clones existed for most of the traits studied. Seed per raceme, which is a function of percent seed set and the number of florets per raceme was closely associated to seed yield and should be a useful tool when screening for high seed yielding plants. Stems per plant were positively associated with percent protein, indicating that plants with large vegetative skeletons were better able to supply developing seeds with the necessary nutrients for protein production. A positive non-significant relationship was found between seed yield and percent protein, suggesting that selection for high seed yield should not adversely affect the protein content of the seed. Heritability estimates obtained by parent-progeny correlations were lower than those obtained by regression of offspring on the female parent; however, both estimators indicated a high degree of association between parents and progeny for stems per plant, racemes per stem, seed weight and percent protein. Lower heritability estimates were obtained for racemes per plant, seed per raceme, percent seed set and seed yield.

The second experiment included one swine and two rat feeding trials to evaluate sainfoin seed as a source of protein for monogastric animals. Sainfoin seed had approximately 36% crude protein and its essential amino acid composition was similar to soybean meal. Weanling pigs performed better on soybean meal than on sainfoin seed; however, weanling rats on 20% protein diets performed equally well on sainfoin seed and soybean meal. When the protein content of the diets was restricted to 11% protein, weanling rats fed sainfoin seed gained as well as rat's fed soybean meal; however, they did not utilize the protein quite as efficiently as rats fed soybean meal or casein. The assay for trypsin inhibitor revealed that raw sainfoin was high in inhibitory activity, and that this activity was drastically reduced or nullified when the seed was autoclaved. Performance and pancreas data indicated the inhibitor did not have a detrimental effect on the feed value of sainfoin seed. Pancreases from rats fed raw sainfoin were not enlarged.

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Approved:

K C Feltner
Head, Major Department

Clee S Cooper
Chairman, Examining Committee

H Goering
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

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ABSTRACT

Yield and yield components of sainfoin seed, and protein quality of sainfoin seed were studied in separate experiments. The first experiment was a study of the components of seed yield in sainfoin and their heritabilities. Components studied included stems per plant, racemes per plant, racemes per stem, florets per raceme, seed per raceme, percent seed set, weight per 100 seeds, and seed yield. Crude protein content of the seed, plant height and lodging were also studied. Significant differences between clones existed for most of the traits studied. Seed per raceme, which is a function of percent seed set and the number of florets per raceme was closely associated to seed yield and should be a useful tool when screening for high seed yielding plants. Stems per plant were positively associated with percent protein, indicating that plants with large vegetative skeletons were better able to supply developing seeds with the necessary nutrients for protein production. A positive non-significant relationship was found between seed yield and percent protein, suggesting that selection for high seed yield should not adversely affect the protein content of the seed. Heritability estimates obtained by parent-progeny correlations were lower than those obtained by regression of offspring on the female parent; however, both estimators indicated a high degree of association between parents and progeny for stems per plant, racemes per stem, seed weight and percent protein. Lower heritability estimates were obtained for racemes per plant, seed per raceme, percent seed set and seed yield.

The second experiment included one swine and two rat feeding trials to evaluate sainfoin seed as a source of protein for monogastric animals. Sainfoin seed had approximately 36% crude protein and its essential amino acid composition was similar to soybean meal. Weanling pigs performed better on soybean meal than on sainfoin seed; however, weanling rats on 20% protein diets performed equally well on sainfoin seed and soybean meal. When the protein content of the diets was restricted to 11% protein, weanling rats fed sainfoin seed gained as well as rats fed soybean meal; however, they did not utilize the protein quite as efficiently as rats fed soybean meal or casein. The assay for trypsin inhibitor revealed that raw sainfoin was high in inhibitory activity, and that this activity was drastically reduced or nullified when the seed was autoclaved. Performance and pancreas data indicated the inhibitor did not have a detrimental effect on the feed value of sainfoin seed. Pancreases from rats fed raw sainfoin were not enlarged.

INTRODUCTION

Most legume varieties have been released for their high forage yielding ability with little consideration being given to seed yield. As a result, many of the current legume varieties have poor seed yields, and breeders are beginning to emphasize seed yield in their breeding programs. 'Uinta', for example, is a variety of alfalfa (*Medicago sativa* L.) that was bred for both high seed and forage yields. This variety is similar to the variety 'Ranger' in forage yield, but produces sixty-seven percent more seed than does Ranger (50).

Sainfoin (*Onobrychis viciaefolia* Scop.) is a newly reintroduced forage legume that is gaining popularity in the Northern Rocky Mountain States and in Canada. Varietal release in this crop has been from selection within and among adapted plant introductions. Although the seed yields of sainfoin have been relatively good, it was felt selection pressure should be placed on its seed yielding ability in the early stages of the breeding program in order to circumvent the problem that has arisen in other legume species.

Seed yield is generally recognized to be a very complex character. It is the end result of the activities of the plant acting within its genetic potential in a given environment. When one or more environmental factors vary, differences in yield may result. Seed yield differences have also been noted among sainfoin varieties and experimental lines grown under the same environmental conditions. These differences indicate that seed yield is also controlled by genetic factors. Since seed yield is a complex character resulting from the interaction of a number of separate characters. yield components should be studied separately, as well as in combination with each other. Knowledge of the components of seed yield and their relative importance can aid plant breeders in selecting superior genotypes based on their phenotypic appearance and behavior, and to determine which component or components should be subjected to the greatest selection pressure for most rapid progress.

The objectives of Experiment I were to: 1) study the components of seed yield in sainfoin; 2) determine their relationships with each other; 3) determine their heritabilities; and 4) determine, if possible, which components were the best indices of seed yield under the environmental conditions found at Bozeman, Montana.

A second series of experiments were initiated to investigate the possible use of sainfoin seed as a protein supplement. Sainfoin seed has 36% protein, and seed yields of this plant are high enough that it might be economically grown as a protein supplement.

Protein supplement is the most expensive ingredient in livestock rations. Soybean meal is commonly used as a protein supplement because of its high protein content and essential amino acid balance.

It is one of the best quality plant proteins available for livestock feeding.

In 1963, soybean meal sold commercially for $83 a ton. In June of 1972, the cost of soybean meal had risen to $131 a ton and in August of 1973, the cost had risen to $334 a ton. These rapidly rising costs have caused investigators to look for other sources of protein.

Woodman and Evans (65) reported lambs did very well on sainfoin seed, and suggested the lambs would have performed better if the seed pod had been removed. Holden (25) found that rats fed sainfoin seed had higher feed consumptions and lower feed efficiencies than rats fed soybean meal. He surmised that sainfoin seed with the pod removed would compare favorably with soybean meal.

The purpose of Experiment II was to compare milled sainfoin seed with soybean meal as a source of protein for monogastric animals.

REVIEW OF LITERATURE

Morphology and Types

Sainfoin is a long-lived, deep rooted perennial (51). The root system consists of a main tap root with several large and numerous fine lateral roots (58). Tap roots may be 5 cm in diameter (51), and extend to a depth of 1-10 m (2,58). Most nodules occur on the fine lateral roots, but a few may also occur on the young tap root (58).

Sainfoin has a branched crown from which numerous erect stems arise. Leaves, born on a petiole, are pinnately compound with 11-29 leaflets per leaf (51,58). The inflorescence is born on an erect raceme with 5-80 florets (6,51). The seed is born in single-seeded pods which are brown, indehiscent, lenticular and reticular on the surface (51). The seed is kidney shaped, with the hilum situated in the middle of the concave edge. It is 2.5 mm long, 2.0-3.5 mm wide and 1.5-2.0 mm thick. Seed color ranges from olive to brown or black (58). The weight of 1000 milled seeds ranges from 13.2-16.8 g (58), and the seed pod comprises approximately 30% of the seed weight of unmilled seed (6).

Sainfoin consists of several types with differing growth and adaptation characteristics. In Russia the three types generally grown are common, sand, and transcaucus. Common sainfoin is distributed in the forest-steppe belt of the Ukraine. It has moderate drought tolerance and winterhardiness, slow recovery after cutting, and is used as a one-cut crop. Sand sainfoin is also adapted to the Ukraine. It has excellent drought tolerance and winterhardiness, and its one-cut growth habit resembles that of common. Transcaucus sainfoin is grown in the Transcaucasus region, and exceeds common sainfoin in winter-hardiness, drought tolerance and yield. It grows rapidly, has good recovery after harvesting, and gives 2-3 cuttings a year under irrigation. The transcaucus type is shorter-lived than common sainfoin (2,55).

Common and Giant are the two types of sainfoin grown in Great Britain. Common sainfoin has limited stem elongation the year of establishment, and rarely flowers until the second year. The stems are 60-90 cm long at flowering, and are decumbent at the base. The leaves of Common are smaller and have fewer leaflets than Giant. Common sainfoin produces one hay crop a year. Giant is a multicut, short-lived sainfoin. Stem elongation and flowering occur the first year. The stems of Giant are longer and less decumbent than Common. Two hay crops are usually obtained from Giant (28).

History

Sainfoin is not a new crop. Shain (55) reported that sainfoin was used as a forage crop in Russia over 1,000 years ago. Four hundred years ago sainfoin gained recognition in southern France, where it was first cultivated in 1582, and its culture first described in 1629. It was grown in Germany in the 17th Century and in Italy in the 18th Century (51). Its use in Russia and Europe was primarily on dry calcareous soils where other forage legumes did not thrive (2,51,55,58).

Sainfoin's popularity in Europe and Russia has been attributed to its drought tolerance, winterhardiness, disease resistance, forage quality and its non-bloating characteristics (2,11,51,58).

Although sainfoin is known to have been grown in North America prior to 1900, it gained little recognition as a forage crop until 1964 when the variety 'Eski' was released by the Montana Agricultural Experiment Station (14). Eslick (13) attributed sainfoin's slow rise in popularity to its being tested on soils where it was not adapted, overlooking its non-bloating characteristics, and to investigators being misled by visual notes on coarseness, leafiness and probable palatability. Since the release of Eski, sainfoin has been evaluated in most of the western states, and breeding programs have been initiated in New Mexico and intensified in Canada.

Sainfoin for Hay or Pasture

Alfalfa (*Medicago sativa* L.) has long been the major forage legume in Montana. In recent years, its production was limited by alfalfa weevil infestations. This caused many growers to look for alternative hay crops. Sainfoin is resistant to the alfalfa weevil which suggested that it might be a suitable alternate (4,7,62).

As a hay crop, sainfoin's performance has been variable. It has yielded less than, the same as, and more than alfalfa, depending on the location and environmental conditions (7,19,42,52). In general, sainfoin has yielded more in the first cutting and less in the second cutting than alfalfa (7). Yields of sainfoin have been reduced in the third and fourth years due to a reduction in stand. This reduction in stand has been attributed to root and crown rot organisms (7,38). Persistence is also poor under irrigation or on soils with high water tables (2,7,13,55).

The nutritive value of sainfoin hay is high. It is lower in crude protein, crude fiber and ash than alfalfa, but is higher in nitrogen free extract (27). Cattle feeding trials in Nevada have shown sainfoin to be equal to alfalfa for average daily gains, feed consumption, feed efficiency and disgestability (27), and swine feeding trials in Montana have indicated that 3% ground sainfoin in the diet is equivalent to 3% ground alfalfa in the diet (44). Thus, sainfoin may be a suitable hay crop for areas not suited for alfalfa.

Pasture trials have shown that sainfoin is a very palatable and nutritious non-bloating legume. Cattle and sheep have shown a definite preference for sainfoin over alfalfa, cicer milkvetch (Astrogulus cicer L.), birdsfoot trefoil (Lotus corniculatus L.) and crested wheatgrass (Agropyron desertorum Fisch.)(20,24,63). Yearling steers grazed on sainfoin had superior gains and beef production over steers grazed on other pastures, although the stocking rate of sainfoin was lower. The lower stocking rate of sainfoin was attributed to the uneven seasonal distribution of forage from the variety Eski. In May and June, forage production of Eski is very high, but later in the season it is low. Consequently, the stocking rate had to be varied from 6-8 to 2-4 animals per hectare (32,33). The variety 'Remont', a multicut variety released in 1971, has a seasonal yield distribution similar to alfalfa which should help to alleviate this problem (5).

Seed Production

Sainfoin seed production has been relatively good. It has been estimated the average yield of sainfoin seed in Montana is 500 kg per hectare (4). Seed yields have been reported in excess of 700 kg per hectare in Nevada (27), 1,000 kg per hectare in Idaho (42) and Canada (19), and 1,300 kg per hectare in Montana (8).

Cultural practices. Uniform, firm and well prepared seed beds are essential to obtaining good stands. On most soils, a planting depth of one-half inch has provided satisfactory results. Despite its large seed size, sainfoin does not emerge well from deep plantings (19,27).

Significant responses have been obtained from the use of N fertilizer on sainfoin for both forage yield (27,56) and seed yield (56).

It has not been shown to respond to P fertilization (27,53,56). Sainfoin's response to N fertilization has been attributed to the lack of an effective N fixing rhizobia (3,19,27,41,56).

In alfalfa, maximum seed yields have been obtained from plants that were slightly water stressed during flowering and seed set. An irrigation at early bloom was sufficient to restore moisture to the root zone and provide enough moisture to mature the crop. Irrigation at the time of full bloom stimulated vegetative growth and decreased seed yields (9,59,60,61). Although the effect of soil moisture on sainfoin seed production has not been studied, irrigation during flowering and seed set have not been observed to stimulate vegetative growth and was not felt to be detrimental to seed yield (64).

There has been general recognition among alfalfa investigators that lower plant densities result in higher seed yields. Planting rates of 1-2 kg per hectare in 60 cm rows, or 3-5 kg per hectare broadcast resulted in the highest alfalfa seed yields (9,30,31,39).

Plant density-seed yield studies in sainfoin have not been in agreement. Jenson and Sharp (27) reported the highest seed yields were obtained with 90 cm row spacings and the lowest with 15-30 cm row spacings. Seeding rates from 2-10 kg per hectare had no appreciable effect on seed yields. Hanna et al. (19) obtained maximum seed yields from sainfoin planted in 60-90 cm rows seeded at the rate of 6-9 kg per hectare. Carleton and Wiesner (6), however, obtained maximum seed yield from sainfoin seeded in 7 cm rows. Increasing row spacing to 15 cm decreased seed yields 50%. Seeding rates did not appreciably affect yield. These investigators attributed sainfoin's higher seed yield at narrow row spacings to sainfoin's inflorescence being born at the apex of an erect stem which resulted in maximum exposure of the inflorescence to pollinating insects, and to an increased number of inflorescences per unit area (6).

The common recommendation for harvesting alfalfa for seed is when one-half to two-thirds of the pods are brown to black (40,48,61). In Canada, it was recommended that sainfoin be cut for seed when the pods at the base of the raceme have turned brown (19). Montana data indicates that the relationship of pod color to seed maturation varies with locations and environmental conditions. Maximum seed yields were obtained when the seed was harvested at 40% moisture. This moisture percentage occurred when 10, 33, and 65% of the pods were brown at Moccasin, Bozeman, and Kalispell, Montana, respectively. Earlier harvests, prior to 40% moisture, resulted in poor quality seed, and delayed harvests resulted in seed loss due to shattering. These investigators recommended that sainfoin be swathed at 40% moisture, allowed to dry in the windrow before threshing, and that the seed be air-dried to 12% moisture prior to storage (8).

<u>Components of seed yield</u>. Seed yield is a function of the number of seeds per unit area and seed weight (49). Seeds per unit area may be further subdivided into its individual components which include: plants per unit area, stems per plant, racemes per stem, florets per raceme, and percent seed set.

Seeds per raceme, percent seed set (used as a measure of self- and cross-fertility) and seed weight have been studied in sainfoin. Hanna (18) reported cross-fertility ranged from 3.7-78.3% with a mean of 35.6%, and self-fertility ranged from 0-21.4% with a mean of 4.8%. Self- and cross-fertility were highly correlated (r = .65**). Carleton and Wiesner (6) found a positive relationship between cross-fertility and seed yield, and a negative relationship between seeds per raceme and seed weight. They stated selection for large seeded plants could result in selection for low cross-fertility.

In alfalfa, a number of components and traits have been studied to determine which may be used as indices of seed yield. Factors studied include date of initial bloom, stems per plant, racemes per stem, raceme length and width, florets per raceme, flower color, plant height, forage yield, self- and cross-fertility, seeds per pod, curls per pod, and seed weight per plant. The importance of these factors varied with locations and environmental conditions. None have been universally accepted as good indices of seed yield in alfalfa, although one or more may be considered indicative of seed yield at a particular location (45,54).

Sainfoin Seed as a Protein Supplement

In 1947, Woodman and Evans (65) compared the chemical composition and nutritive value of rye grass, clover, alfalfa and sainfoin seed meal. They indicated that lambs fed unmilled sainfoin seed performed well and would have equalled the gains of lambs fed alfalfa and clover seed meal if the seed pod had been removed.

Holden (25) compared the protein quality of unmilled sainfoin seed and pigweed seed with soybean meal in rat feeding trials. He reported no significant difference in weight gain of rats fed sainfoin seed and soybean meal, although rats fed sainfoin seed consumed more feed and had lower feed efficiencies than rats fed soybean meal. He surmised milled sainfoin seed would compare favorably with soybean meal as a source of protein.

For many years commercial soybean meal has been recognized to be an excellent protein supplement for livestock and has recently been used in increasing quantities as a protein source for humans. Soybean meal's popularity as a protein source may be attributed to its high protein content and essential amino acid balance. Research has indicated that the most limiting amino acid was methionine (22), but even without supplemental methionine, soybean meal is one of the best quality plant proteins available (45).

In 1936, Hayward, Steenbock, and Bohstedt (23) determined that the heating process associated with commercial extraction of oil from soybeans resulted in a definite improvement in the feeding value of soybean meal. Recent research indicates the poor feeding quality of raw soybean meal may be attributed to the presence of heat labile trypsin inhibitors and a deficiency of sulfur containing amino acids (17, 28,31,36,66). The inhibitors present in unheated soybean meal results in hypertrophy of the pancreas, increased synthesis of trypsin and chymotrypsin, and decreased synthesis of amylase (17,31,36). Hypertrophy of the pancreas is detectable in four days (17). Excess levels of trypsin and chymotrypsin are lost to the animals through excretion. These enzymes are high in sulfur containing amino acids and their excretion is thought to create a deficiency for these amino acids. In addition, the inhibitors have a disproportionate amount of cystine, which is not available to the animal and may accentuate deficiencies (28).

Although heating soybean meal has been shown to be an effective means of inactivating the activity of these inhibitors, there is also evidence that genetic variability exists in soybeans for decreased trypsin inhibitor activity. Yen (66) reported that rats fed experimental line 661 excreted less fecal nitrogen, retained more nitrogen, had better gains, and had smaller pancreases than rats fed Clark and Harsoy varieties. Although 661 was superior to the other two varieties, it was inferior to heated soybean meal.

METHODS AND MATERIALS EXPERIMENT I

Ten clones were selected for study on the basis of high 1967 seed yields in a space-planted nursery. Seed yield of these clones ranged from 248 to 391 g. Clonal propagules (So) and open-pollinated (OP) progeny from these ten clones were used to study the components of seed yield in sainfoin and their heritabilities.

In the fall of 1970, crown bud cuttings and seedlings from OP seed from these clones were started and maintained individually in the greenhouse. In the spring of 1971, they were transplanted to the field. A split plot randomized complete block design with three replications was used. Clones were assigned to mainplots and generations (So,OP) assigned to subplots. The rows were 3 m long and .6 m apart. Each row contained 9 plants.

The plants became established during the 1971 growing season. Data were collected in 1972 on stems per plant, racemes per plant, racemes per stem, florets per raceme, seed per raceme, percent seed set, weight per 100 seeds, seed yield, crude protein content of the seed, plant height, and lodging.

Stems per plant were obtained by selecting three plants at random in each row and counting the number of basal stems. The total number of racemes on each of these plants were also counted to obtain racemes per plant. Racemes per stem were derived by dividing racemes per plant by stems per plant.

Florets per raceme were determined by randomly selecting ten racemes per row and counting the number of florets on each raceme. These racemes were tagged and the number of florets counted recorded on the tag. Prior to harvesting for seed, the tagged racemes were collected and the number of seeds per raceme counted. Percent seed set was calculated by dividing seeds per raceme by the number of florets that had been available for pollination and multiplying by 100.

Seed yield was obtained by hand harvesting the plants in each row and weighing the cleaned seed to the nearest g. A random sample of 100 seeds from the plants in each row was weighed to the nearest mg to obtain weight per 100 seeds. Crude protein was determined by Kjeldahl method (N x 6.25)(1) from another random sample of seed from the plants in each row by the Chemistry Station Analytical Laboratory at Montana State University. The analyses were performed on seed contained in the pod.

Plant height was determined and lodging was estimated just prior to seed harvest. Plants were measured, to the nearest cm, from the base of the crown to the tip of the longest stem at three randomly selected points along a row. Lodging was estimated on a one to five scale. Rows with all of the plants standing erect were rated one, and rows with all of the plants prostrate were rated five.

Analysis of variance was used to detect significant differences for each of the traits studied. Row means for each of the characters were used for these analyses. When the F test indicated significance, Duncan's New Multiple Range Test was used to separate means (12). Narrow sense heritability estimates were determined for traits in which significant differences existed by parent-progeny correlations (15) and by regression of OP on So (35). All possible simple correlations between traits were also obtained for the So and OP generations.

Good growing conditions prevailed during this experiment. The field was sprinkler irrigated as necessary to keep the soil moist and allow the plants to grow vigorously. Irrigation was stopped when plants were in the early pod stage to minimize seed loss through shattering. The plants were sprayed at weekly intervals with malathion from early bloom to full bloom to minimize insect damage, particularly from Lygus spp. Although beehives were not placed around the field, honeybees were abundant when the plants were blooming.

RESULTS AND DISCUSSION EXPERIMENT I

Seed yield is a function of the number of seeds per unit area and seed weight. The number of seeds per unit area may be further subdivided into its individual components, i.e., stems per unit area, racemes per stem, florets per raceme, and percent seed set. This study was designed to determine which components were good indices of seed yield in sainfoin, their relationship to each other, and the heritability of each of these components under the environmental conditions at Bozeman, Montana in 1972.

Stems Per Plant

Clones differed significantly for the number of stems per plant (Table 1). Clones A-33 and A-67 had the greatest number of stems per plant with 57.9 and 55.2, respectively. Clone A-40 had the fewest stems per plant with 31.6. Clone A-63, which had the highest seed yield, was intermediate for this character. Significant differences between generations were not detected, and the clone x generation interaction was not significant.

Stems per plant of the So generation were negatively correlated (Table 2) with racemes per stem (r = -.71*), and positively correlated with crude protein content of the seed (r = .76*). Stems per plant of the OP progeny were negatively correlated with racemes per stem (r = -.74*) and positively correlated with percent seed set (r = .66*).

Table 1. Clone and Generation Means for Number of Stems Per Plant

Clone	Generation	Generation mean	Clone mean
A-33	So	66.0	57.9 a[1]
	OP	49.9	
A-67	So	58.1	55.2 a
	OP	52.2	
A-34	So	54.6	49.4 b
	OP	44.3	
A-93	So	51.3	48.7 b
	OP	46.0	
A-10	So	48.2	47.6 b
	OP	47.0	
A-63	So	47.9	46.1 bc
	OP	44.6	
A-55	So	43.0	42.8 c
	OP	42.6	
A-54	So	44.8	42.2 c
	OP	39.6	
A-70	So	33.1	37.9 d
	OP	42.7	
A-40	So	31.6	31.6 e
	OP	31.6	
Mean	So	47.9	
	OP	44.1	

1 - Means in the same column followed by different letters differ significantly ($P<.05$).

Stems per plant were not significantly correlated with any of the other traits studied, including seed yield (Table 2).

Table 2. So, OP, and Total Population Correlation Coefficients for Stems/Plant and Other Traits Studied.

Component	So	OP
Racemes/plant	.41	.41
Racemes/stem	-.71 *	-.74 *
Florets/raceme	-.38	-.10
Seed/raceme	-.06	.52
% Seed set	.14	.66 *
Seed yield	-.07	.28
Wt/100 seed	-.27	-.33
% Protein	.76 *	.51
Plant height	.10	-.23
Lodging	.40	.42

* - P<.05

These data indicate that differences among clones exist for stems per plant, but that this character is not indicative of seed yield. Plants with many stems had fewer racemes per stem, but the total number of racemes per plant was not significantly affected by this relationship (r = .41). Increased stems per plant were also associated with increased protein content in the seed for the So generation. This association was not found for the OP generation (Table 2), although the correlation coefficient did approach significance (r = .51). These relationships suggest that plants with a large vegetative skeleton are able to supply developing seed with the essential nutrients for protein production.

Stems per plant of the OP progeny were significantly related to percent seed set. The meaning of this relationship is not known. It is possible that OP progeny with more stems had less natural stripping of florets, increased pollination, or less shattering. Any of these factors can significantly affect percent seed set. It is also possible that this is a chance relationship and has no biological significance.

<u>Racemes Per Plant</u>

Significant differences occurred among clones and between generations for the number of racemes per plant; however, the clone x generation interaction was significant, negating meaningful interpretation of main effects (Table 3). This interaction resulted from the So of clones A-33 having more racemes per plant than the OP progeny, and the OP progeny of clones A-54, A-70, and A-93 having more racemes per plant than the So's. Differences among generations within the other clones studied were not significant.

Racemes per plant of the So generation were positively correlated (r = .70*) with protein content of the seed (Table 4), whereas racemes per plant of the OP progeny were associated (r = .68*) with

Table 3. Clone and Generation Means for the Number of Racemes Per Plant.

Clone	Generation	Generation mean	Clone mean
A-55	So	251.6 a[1]	240.3 a[2]
	OP	229.1 a	
A-34	So	233.1 a	227.7 ab
	OP	222.3 a	
A-67	So	212.8 a	221.7 b
	OP	230.7 a	
A-70	So	193.9 b	220.6 b
	OP	247.3 a	
A-33	So	242.8 a	220.5 b
	OP	198.2 b	
A-10	So	206.4 a	218.3 b
	OP	230.1 a	
A-63	So	222.0 a	210.0 b
	OP	198.0 a	
A-54	So	160.7 b	189.2 c
	OP	217.7 a	
A-93	So	149.3 a	170.7 d
	OP	192.0 b	
A-40	So	165.3 a	169.0 d
	OP	172.7 a	
Mean	So	208.8 b[3]	
	OP	213.8 a	

1 - Generation means within a clone followed by different letters differ significantly (P<.05).

2 - Means in the same column followed by different letters differ significantly (P<.05).

3 - Generation means followed by different letters differ significantly (P<.05).

a higher incident of lodging. Racemes per plant were not correlated with seed yield.

Table 4. So, OP, and Total Population Correlation Coefficients for Racemes Per Plant and Other Traits Studied.

Component	So	OP
Stems/plant	.41	.41
Racemes/stem	.34	.28
Florets/racemes	.14	.30
Seed/racemes	.22	.10
% Seed set	.25	-.04
Seed yield	.49	-.11
Wt/100 seed	-.47	-.45
% Protein	.70 *	.29
Plant height	.30	-.27
Lodging	.16	.68 *

* – P<.05

Racemes Per Stem

Clones A-70, A-55, and A-40 had the highest number of racemes per stem, and clones A-67, A-33 and A-93 had the least (Table 5). Clones A-34, A-10, A-63, and A-54 were intermediate for this character. When measured across all clones, the OP progeny had significantly more racemes per stem than the So's. The clone x generation interaction was not significant.

Table 5. Clone and Generation Means for the Number of Racemes Per Stem.

Clone	Generation	Generation mean	Clone mean
A-70	So	5.8	5.8 a[1]
	OP	5.7	
A-55	So	6.0	5.7 a
	OP	5.4	
A-40	So	5.3	5.4 a
	OP	5.6	
A-34	So	4.5	4.7 b
	OP	5.0	
A-10	So	4.4	4.6 b
	OP	4.9	
A-63	So	4.6	4.5 b
	OP	4.5	
A-54	So	3.7	4.4 b
	OP	5.1	
A-67	So	3.6	4.0 c
	OP	4.3	
A-33	So	3.7	3.9 c
	OP	4.1	
A-93	So	3.1	3.6 c
	OP	4.2	
Mean	So	4.5 b[2]	
	OP	4.9 a	

1 - Means in the same column followed by different letters differ significantly (P<.05).

2 - Generation means followed by different letters differ significantly (P<.05).

Racemes per stem were negatively correlated with stems per plant for the So (r = -.71*) and OP generations (r = -.74*), but were not correlated with any other trait (Table 6). These correlations suggest a negative relationship between racemes per plant and vegetative growth. The number of racemes per plant did not affect seed yield.

Table 6. So, OP, and Total Population Correlation Coefficients for Racemes Per Stem and Other Traits Studied.

Component	So	OP
Stems/plant	-.71 *	-.74 *
Racemes/plant	.34	.28
Florets/raceme	.49	.39
Seed/raceme	.15	.35
% Seed set	-.04	-.61
Seed yield	.23	-.35
Wt/100 seed	-.10	-.02
% Protein	-.24	-.40
Plant height	.13	-.05
Lodging	-.24	.04

* - P<.05

Florets Per Raceme

Clone A-10 had the most florets per raceme with 62.27. Clones A-93, A-67, and A-33 had the fewest with 54.05, 53.33, and 52.98, respectively (Table 7). Generations had similar numbers of florets per raceme. The clone x generation interaction was significant and occurred because the OP progeny of clone A-63 had fewer florets per raceme than the So's. Significant differences between generations of the other clones were not detected.

Florets per raceme were associated with the number of seed per raceme for the So's (r = .70*) (Table 8). Florets per raceme of the OP progeny were not significantly correlated with any of the traits studied. These data indicate that clones differ in the number of florets per raceme but that this component was not indicative of seed yield.

Seed Per Raceme

Seeds per raceme differed significantly among clones and between generations (Table 9). The clone x generation interaction was significant, negating meaningful interpretation of main effects. This interaction occurred because the OP progeny of clones A-63, A-34, and A-54 had fewer seed per raceme than the So's. Differences between generations of the other clones were not significant.

Seeds per raceme of the So's were correlated (Table 10) with florets per raceme (r = .70*), percent seed set (r = .91**), and seed yield (r = .71*). Seeds per raceme of the OP progeny were associated with percent seed set (r = .89**) and seed yield (r = .70*). Seed

Table 7. Clone and Generation Means for the Number of Florets Per Raceme.

Clone	Generation	Generation mean	Clone mean
A-10	So	65.8 a[1]	68.3 a[2]
	OP	70.7 a	
A-63	So	69.4 a	65.6 b
	OP	61.8 b	
A-70	So	64.6 a	63.6 c
	OP	62.6 a	
A-34	So	65.8 a	62.1 cd
	OP	58.4 a	
A-55	So	63.4 a	61.4 d
	OP	59.4 a	
A-54	So	61.9 a	59.2 e
	OP	56.6 a	
A-40	So	56.4 a	58.1 e
	OP	59.8 a	
A-93	So	52.1 a	54.0 f
	OP	56.0 a	
A-67	So	51.7 a	53.3 f
	OP	55.0 a	
A-33	So	49.3 a	53.0 f
	OP	56.3 a	
Mean	So	60.0	
	OP	59.7	

1 - Generation means within a clone followed by different letters differ significantly (P<.05).

2 - Means in the same column followed by different letters differ significantly (P<.05).

Table 8. So, OP, and Total Population Correlation Coefficients for Florets Per Raceme and Other Traits Studied.

Component	So	OP
Stems/plant	-.38	-.10
Racemes/plant	.14	.30
Racemes/stem	.49	.39
Seed/raceme	.70 *	.54
% Seed set	.34	.10
Seed yield	.32	.54
Wt/100 seed	-.45	-.03
% Protein	-.19	-.53
Plant height	.60	.15
Lodging	.06	.41

* - P<.05

Table 9. Clone and Generation Means for the Number of Seed Per Raceme.

Clone	Generation	Generation mean	Clone mean
A-10	So	29.6 a[1]	27.6 a[2]
	OP	25.5 a	
A-63	So	34.1 a	26.3 a
	OP	18.6 b	
A-70	So	22.5 a	21.3 b
	OP	20.1 a	
A-34	So	25.8 a	21.0 b
	OP	16.1 b	
A-67	So	20.2 a	20.6 b
	OP	21.1 a	
A-33	So	19.1 a	20.2 b
	OP	21.2 a	
A-40	So	21.4 a	19.6 bc
	OP	17.8 a	
A-54	So	23.1 a	18.2 c
	OP	13.3 b	
A-93	So	14.1 a	16.8 d
	OP	19.6 a	
A-55	So	17.7 a	16.2 d
	OP	14.7 a	
Mean	So	22.8 a[3]	
	OP	18.8 b	

1 - Means in the same column followed by different letters differ significantly ($P<.05$).

2 - Generation means within a clone followed by different letters differ significantly ($P<.05$).

3 - Generation means followed by different letters differ significantly ($P<.05$).

per raceme of the OP progeny were not correlated with florets per raceme, although the correlation coefficient approached significance (r = .54).

These data suggest the number of seed per raceme is indicative of seed yield and two of its components, florets per raceme, and percent seed set. Seed per raceme should be a useful selection trait when screening for high seed yielding plants.

Table 10. So, OP, and Total Population Correlation Coefficients for Seed Per Raceme and Other Traits Studied.

Component	So	OP
Stems/plant	-.06	.52
Racemes/plant	.22	.10
Racemes/stem	.15	-.35
Florets/raceme	.70 *	.54
% Seed set	.91 **	.89 **
Seed yield	.71 *	.70 *
Wt/100 seed	-.50	-.08
% Protein	-.10	-.12
Plant height	.47	-.22
Lodging	-.19	.08

* - P<.05

** - P<.01

Percent Seed Set

Clones and generations differed significantly for percent seed set (Table 11). The clones x generation interaction, however, was significant, which negated meaningful interpretation of main effects. This interaction resulted because the OP progeny of clone A-63, A-34, and A-54 had fewer seed per available floret than the So's.

Percent seed set for the So's was correlated with seed per raceme and seed yield (Table 12). Percent seed set of the OP progeny was associated with stems per plant and seed per raceme. These data revealed that So plants which set more seeds per available floret have more seed per raceme and higher seed yields. OP progeny that set more seed per available floret also had more seed per raceme. The correlation coefficient for percent seed set and seed yield approached significance.

The average percent seed set for this population of sainfoin was 34.3%. With ideal environmental conditions, percent seed set is a good measure of fertility. Ideal conditions are rarely encountered in the field, and it is doubtful the true fertility level of sainfoin is only 34%. Factors such as lack of pollination, natural flower drop, insect damage, and shattering also affect percent seed set. Visual observations indicate shattering is a very important factor affecting percent seed set in sainfoin. Percent seed set probably reflects shatter resistance better than fertility.

Table 11. Clone and Generation Means for Percent Seed Set.

Clone	Generation	Generation mean	Clone mean
A-10	So	44.97 a[1]	40.49 a[2]
	OP	36.01 a	
A-63	So	49.06 a	39.59 a
	OP	30.12 b	
A-67	So	38.90 a	38.56 a
	OP	38.23 a	
A-33	So	38.88 a	38.18 a
	OP	37.48 a	
A-40	So	37.96 a	33.93 b
	OP	29.20 a	
A-34	So	39.05 a	33.52 bc
	OP	27.99 b	
A-70	So	34.76 a	33.52 bc
	OP	32.28 a	
A-93	So	26.73 a	30.68 cd
	OP	34.62 a	
A-54	So	35.92 a	29.71 de
	OP	23.50 b	
A-55	So	27.92 a	26.33 e
	OP	24.77 a	
Mean	So	37.4 a[3]	
	OP	31.5 b	

1 - Generation means within a clone followed by different letters differ significantly ($P<.05$).

2 - Means in the same column followed by different letters differ significantly ($P<.05$).

3 - Generation means followed by different letters differ significantly ($P<.05$).

Table 12. So, OP, and Total Population Correlation Coefficients for Percent Seed Set and Other Traits Studied.

Component	So	OP
Stems/plant	.14	.66 *
Racemes/plant	.25	-.04
Racemes/stem	-.04	-.61
Florets/raceme	.34	.09
Seed/raceme	.91 **	.89 **
Seed yield	.78 **	.53
Wt/100 seed	-.43	.08
% Protein	.01	.14
Plant height	.25	.36
Lodging	-.30	-.13

* - P<.05

** - P≤.01

Estimates of percent seed set and the reduction in percent seed set due to shattering may be obtained by tagging several racemes with the number of florets present; collecting one-half of the tagged racemes when the seed pods are green and immature; and collecting the other one-half of the tagged racemes at seed maturation (40% moisture). Percent seed set calculated from racemes with immature pods should give an accurate reflection of true percent seed set or fertility. Percent seed set calculated from racemes with mature pods would estimate actual seed set at harvest time, and the differences in the two estimates should reflect losses due to shattering. An experiment such as this should be conducted so that accurate estimates of fertility and seed losses due to shattering may be obtained.

Weight Per 100 Seeds

Weight per 100 seeds differed significantly among clones (Table 13). Clone A-93 had the largest seed (2.80g/hundred), and clone A-34 the smallest (1.98g/hundred). Significant differences in seed weight between generations did not occur, and clones maintained their relative rank for seed weight across generations.

Weight per 100 seeds was not significantly correlated with any of the traits studied for the So's and OP's, including seed yield (Table 14). Negative non-significant relationships occurred between seed weight and most of the factors studied. These data indicate weight per 100 seed is not indicative of seed yield.

Seed Yield

Clones differed significantly for seed yield (Table 15). Seed yield ranged from 158.3 to 325.7g, with clone A-63 having the highest yield. The OP progeny of clone A-93 yielded more seed than did the So generation. Generations of other clones yielded the same.

Seed yield was correlated (Table 16) with seed per raceme for the So (r = .71*) and OP generations (r = .70*) and with percent seed set in the So generation (r = .78**). These data indicated differences

Table 13. Clone and Generation Means for Weight Per 100 Seeds.

Clone	Generation	Generation mean	Clone mean
A-93	So	2.80	2.80 a[1]
	OP	2.80	
A-40	So	2.62	2.56 b
	OP	2.50	
A-55	So	2.63	2.54 b
	OP	2.44	
A-54	So	2.55	2.51 bc
	OP	2.47	
A-63	So	2.45	2.48 bcd
	OP	2.51	
A-70	So	2.45	2.44 cd
	OP	2.42	
A-67	So	2.47	2.42 d
	OP	2.37	
A-10	So	2.45	2.40 d
	OP	2.34	
A-33	So	2.50	2.39 d
	OP	2.29	
A-34	So	1.92	1.98 e
	OP	2.04	
Mean	So	2.5	
	OP	2.4	

1 - Means in the same column followed by different letters differ significantly ($P<.05$).

Table 14. So, OP, and Total Population Correlation Coefficients for Weight Per 100 Seed and Other Traits Studied.

Component	So	OP
Stems/plant	-.27	-.33
Racemes/plant	-.47	-.45
Racemes/stem	-.10	-.02
Florets/raceme	-.45	-.03
Seed/raceme	-.50	-.08
% Seed set	-.43	-.08
Seed yield	-.43	.31
% Protein	-.41	-.53
Plant height	-.29	.35
Lodging	-.28	-.15

Table 15. Clone and Generation Means for Seed Yield.

Clone	Generation	Generation mean	Clone mean
A-63	So	372.7 a[1]	325.7 a[2]
	OP	278.7 a	
A-10	So	222.7 a	255.7 b
	OP	288.7 a	
A-67	So	278.3 a	224.8 bc
	OP	171.3 a	
A-33	So	217.3 a	218.7 c
	OP	220.0 a	
A-40	So	247.0 a	198.1 cd
	OP	149.3 a	
A-34	So	272.3 a	197.3 cd
	OP	123.3 a	
A-54	So	202.7 a	186.3 de
	OP	170.0 a	
A-55	So	213.7 a	178.0 de
	OP	142.3 a	
A-70	So	153.3 a	170.7 de
	OP	188.0 a	
A-93	So	96.3 b	158.3 e
	OP	220.3 a	
Mean	So	227.6	
	OP	195.1	

1 - Generation means within a clone followed by different letters differ significantly ($P<.05$).

2 - Means in the same column followed by different letters differ significantly ($P<.05$).

existed between clones, upon which selection pressure for seed yield may be applied. Seed per raceme, which is a function or percent seed set and florets per raceme, was indicative of seed yield and should be a useful tool in screening for high seed yielding plants.

Table 16. So, OP, and Total Population Correlation Coefficients for Seed Yield and Other Traits Studied.

Component	So	OP
Stems/plant	-.07	.28
Racemes/plant	.49	-.11
Racemes/stem	.22	-.35
Florets/raceme	.32	.54
Seed/raceme	.71 *	.70 *
% Seed set	.78 **	.53
Wt/100 seed	-.43	.31
% Protein	.23	-.12
Plant height	.23	.30
Lodging	-.59	.13

* - P<.05

** - P<.01

<u>Protein Content</u>

Crude protein content of the seed differed significantly among clones (Table 17). Seed of clones A-67 and A-33 had the highest crude protein contents with 26.7 and 26.1%, respectively, and seed of clone

Table 17. Clone and Generation Means for Percent Crude Protein Content of Sainfoin Seed.

Clone	Generation	Generation mean	Clone mean
A-67	So	27.2	26.7 a[1]
	OP	26.1	
A-33	So	26.9	26.1 a
	OP	25.3	
A-34	So	26.4	25.3 b
	OP	24.1	
A-54	So	25.0	25.2 b
	OP	25.3	
A-55	So	26.0	24.8 bc
	OP	23.5	
A-63	So	24.6	24.3 cd
	OP	24.0	
A-70	So	23.7	23.7 de
	OP	23.6	
A-10	So	23.6	23.3 e
	OP	23.0	
A-93	So	23.4	23.1 e
	OP	22.7	
A-40	So	21.7	22.2 f
	OP	22.7	
Mean	So	24.8 a[2]	
	OP	23.0 b	

1 - Means in the same column followed by different letters differ significantly (P<.05).

2 - Generation means followed by different letters differ significantly (P<.05).

A-40 had the lowest with 22.2%. When averaged across all clones, seed of the So generation had a higher crude protein content than seed of the OP progeny. The So's probably had a higher concentration of roots near the soil surface where there was more available nitrogen, and where nodulation was more apt to occur. This would enable the So's to supply developing seed with more nitrogen, and result in a higher protein content. The clone x generation interaction was not significant.

Percent crude protein for the So generation was positively correlated with stems per plant and racemes per plant (Table 18). Percent crude protein content of the OP progeny was not significantly associated with any of the traits studied. Positive non-significant correlation coefficients were obtained for percent protein content and seed yield, thus, a breeding program for increased seed yield should not adversely affect the crude protein content of the seed. Selection for more stems per plant or more racemes per plant may result in increased protein content of the seed.

Plant Height

Clones differed significantly for plant height (Table 19). The tallest clone was A-10 (126.47 cm), and the shortest clone was A-70 (105.47 cm). Differences between generations were not significant.

Plant height was not significantly associated with any of the traits studied (Table 20).

Table 18. So, OP, and Total Population Correlation Coefficients for Percent Crude Protein and Other Traits Studied.

Component	So	OP
Stems/plant	.76 *	-.51
Racemes/plant	.70 *	.29
Racemes/stem	-.24	-.40
Florets/raceme	-.19	-.53
Seed/raceme	-.10	-.12
% Seed set	.01	.14
Seed yield	.23	-.12
Wt/100 seed	-.41	-.53
Plant height	-.07	-.10
Lodging	.32	-.03

* - $P<.05$

Table 19. Clone and Generation Means for Plant Height (cm).

Clone	Generation	Generation mean	Clone mean
A-10	So	131.9	126.5 a[1]
	OP	121.0	
A-55	So	123.6	121.9 b
	OP	120.2	
A-63	So	120.6	121.0 b
	OP	121.4	
A-54	So	114.6	118.9 bc
	OP	123.3	
A-34	So	124.6	116.6 c
	OP	108.7	
A-40	So	115.0	115.7 c
	OP	116.3	
A-93	So	116.1	115.5 c
	OP	114.8	
A-67	So	110.3	111.4 d
	OP	112.4	
A-33	So	112.0	110.9 d
	OP	109.8	
A-70	So	108.3	105.5 e
	OP	102.6	
Mean	So	117.7	
	OP	115.0	

1 - Means in the same column followed by different letters differ significantly ($P<.05$).

Table 20. So, OP, and Total Population Correlation Coefficients for Plant Height and Other Traits Studied.

Component	So	OP
Stems/plant	.10	-.23
Racemes/plant	.30	-.27
Racemes/stem	.13	.05
Florets/raceme	.60	.15
Seed/raceme	.47	-.22
% Seed set	.25	.36
Seed yield	.23	.30
Wt/100 seed	-.29	.35
% Protein	-.07	-.10
Lodging	.06	.26

Lodging

Clones were equally susceptible to lodging (Table 21). When averaged across clones, the So's lodged significantly more than the OP progeny. This may have been due to the So plants being vegetative propagules, whereas the OP progeny were grown from seed. Vegetatively propagated sainfoin has less root system and, as a result, may lodge more readily. Lodging was not associated with any of the other traits studied (Table 22).

Table 21. Clonal and Generation Means for Lodging Rating.

Clone	Generation	Generation mean	Clone mean
A-40	So	1.0	1.5
	OP	2.0	
A-54	So	3.0	3.0
	OP	3.0	
A-63	So	3.7	3.2
	OP	2.7	
A-67	So	3.3	3.3
	OP	3.3	
A-55	So	4.3	3.3
	OP	2.3	
A-33	So	4.0	3.3
	OP	2.7	
A-93	So	4.3	3.7
	OP	3.0	
A-70	So	4.3	3.8
	OP	3.3	
A-34	So	4.3	3.8
	OP	3.3	
A-10	So	3.7	3.8
	OP	4.0	

Table 22. So, OP, and Total Population Correlation Coefficients for Lodging and Other Traits Studied.

Component	So	OP
Stems/plant	.40	.42
Racemes/plant	.16	.68 *
Racemes/stem	-.24	.04
Florets/raceme	.06	.41
Seed/raceme	-.19	.08
% Seed set	-.30	-.13
Seed yield	.59	.13
Wt/100 seed	-.28	-.15
% Protein	.32	-.03
Plant height	.06	.26

* - P<.05

Heritability Estimates for Traits Studied

Heritability estimates (Table 23) were obtained by parent-progeny correlations (heritability = r^2 x 100), and by regression of offspring on female parent (heritability = 2b x 100). Estimates obtained by regression were higher than those obtained by parent-progeny correlations, and three of the regression estimates (racemes per stem, seed weight, and percent protein) were in excess of 100%. These higher estimates could arise from failure to satisfy all of the assumptions on which heritability estimates derived by regression are based. Knipe (29) reported sainfoin to be a cross-pollinated crop that is highly self-fertile. Inbreeding would result in an increase in heritability estimates, when calculated as twice the regression value, because with selfing both the male and female parents are known and heritability is equal to the regression value (35). Deviations from additive gene action could also result in higher heritability estimates (35). Frey and Horner (16) attributed discrepancies in heritability estimates, when calculated by regression, to scaling differences between parents and progeny. Scaling differences are generally associated with a genotype x environment interaction in which the parents are grown in one year and the progeny in another, and the environmental conditions tend to speed up, slow down, increase or decrease the production of some plant characteristics. The parents and progeny in this experiment were grown together, and it is doubtful that scaling differences were a factor.

Heritability estimates are indicative of the progress that may be made through selection, and may also be used as an aid in determining the type of selection program to be used, i.e., high heritability indicates that effective selection on an individual plant basis is possible, whereas low heritability indicates that selection should be based on progeny tests. Although the heritability estimates determined by parent-progeny correlations were lower than those obtained by regression (Table 23), both estimators indicated a high degree of associated between parents and progeny for stems per plant, racemes per stem, seed weight, and percent protein. Rapid progress through a simple mass selection program for these traits should be possible. Lower heritability estimates were obtained for racemes per plant, seed per raceme, percent seed set, and seed yield. Selection for these traits should be based on progeny tests.

Table 23. Heritability Estimates for Traits Studied.

Trait	Method of Determination r^2 x 100	2b x 100
Stems/plant	69	99
Racemes/plant	12	44
Racemes/stem	68	102
Florets/raceme	36	81
Seed/raceme	3	22
% Seed set	4	30
Weight/100 seed	71	151
Seed yield	2	18
% Protein	63	105
Plant height	26	93

The heritabilities of seed yield and the two components most closely associated with it, seed per raceme and percent seed set, were small and of similar magnitude. Thus, it appears one may select for increased seed yield, but that progress will be slow. It is felt the greatest progress can be made by increasing percent seed set. In this study, only 34% of the available florets set seed. It appeared that the greatest loss was from shattering of seed. The possibility of developing shatter resistant lines should be explored as a means of increasing seed yield in sainfoin.

SUMMARY AND CONCLUSIONS EXPERIMENT I

Clonal propagules and open-pollinated progeny from 10 clones were used to study the components of seed yield in sainfoin and their heritabilities. Traits studied included stems per plant, racemes per plant, racemes per stem, florets per raceme, seed per raceme, percent seed set, seed yield, weight per 100 seeds, crude protein content of the seed, plant height and lodging.

Clones differed for most of the traits studied. Seed per raceme, which is a function of percent seed set and the number of florets/ raceme, was closely associated with seed yield and should be a useful tool in screening for high seed yielding plants.

Stems per plant were positively correlated with percent protein, suggesting that plants with large vegetative skeletons were better able to supply developing seeds with the essential nutrients for protein production. A positive non-significant association was found between percent protein and seed yield. Thus, it should be possible to increase seed yield without adversely affecting protein content of the seed.

Heritability estimates were calculated by parent-progeny correlations and by regression of offspring on the female parent. Heritability estimates determined by parent-progeny correlation were lower than those obtained by regression, however, both estimators indicated a high degree of association between parents and progeny for stems per plant, racemes per stem, seed weight, and percent protein. Rapid progress through a simple mass selection program for these traits should be possible. Lower heritability estimates were obtained for racemes per plant, seed per raceme, percent seed set, and seed yield. Selection for these traits should be based on progeny tests.

METHODS AND MATERIALS EXPERIMENT II

Trial I

Four isonitrogenous diets containing 20% protein were fed to weanling pigs to compare the protein quality of milled sainfoin seed with 48.5% protein soybean meal (SBM). The protein supplements for these diets were SBM, SBM with sainfoin oil added, milled sainfoin seed and milled sainfoin seed with the oil extracted.

The sainfoin seed was milled through a hammer mill to remove the pods, and the chaff removed on a clipper cleaner. One-half of the milled sainfoin seed (hereafter referred to as sainfoin seed) was soaked for seven days in hexane to remove the oil. This oil was recovered by distilling the hexane, and was added to one of the SBM rations.

Proximate analyses, calcium and phosphorous determinations were made on the protein sources used, and on the diets fed (1). Percentage composition of the diets fed are in Table 24. Amino acid content of sainfoin seed, with and without oil, and of SBM were determined with a Beckman 120C automatic amino acid analyzer (57).

Twenty-four crossbred weanling pigs weighing approximately 14.3 kg were stratified for sex, initial weight and litter in three replications. They were assigned to pens in groups of four with each pen containing two barrows and two gilts. Diets were assigned at random to pens within a replication. The pens were 1.5 x 2.1 m equipped with self-feeders, automatic cup-type waterers, and located in a heated and ventilated total confinement structure with slotted floors. Weight gain and feed consumption were measured for two 7 day periods and at the end of 18 days. Average daily gains, average daily feed consumption, and feed efficiency ratios were calculated for each pen group within replications and analyzed by the least-square procedures (21).

Table 24. Percentage Composition of Sainfoin and Soybean Meal Diets Fed to Weanling Pigs. Trial I.

	Protein Source			
Ingredients	SBM	SBM + sainfoin oil	Sainfoin	Extracted sainfoin
Wheat	75.50	73.30	66.00	66.60
Soybean meal, 48.5%	20.00	20.60	---	---
Sainfoin	---	---	30.00	---
Extracted sainfoin	---	---	---	29.40
Sainfoin oil	---	1.50	---	---
Monosodium phosphate	1.00	1.10	0.50	0.50
Limestone	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50
Vitamin trace mineral mix[1]	0.75	0.75	0.75	0.75
Antibiotic (ASP-250)[2]	0.25	0.25	0.25	0.25

1 - Vitamin A - 500,000 USP units, Vitamin D_3 - 100,000 IC units, Vitamin E - 1,000 I units, Vitamin B_{12} - 4 mg, Riboflavin - 700mg, Niacin - 4,000 mg, d-Pantothenic Acid - 2,000 mg, choline - 100,000 mg, Zinc - 9,100 mg, Iron - 4,500 mg, Manganese - 2,500mg, Copper - 450 mg, Cobalt - 45 mg, Iodine - 68 mg per 454 g of vitamin-mineral mix.

2 - Furnished 100 g chlorotetracycline, 100 g sulfamethazine and 50 g penicillin per 2.27 kg.

Trial II

Six isonitrogenous, isocaloric diets containing 20% protein and 4% oil were fed to 90 female rats of the Holtzman strain. The average initial weight of the rats fed each diet was approximately 69 g. The protein sources for these diets were: 1) sainfoin seed; 2) sainfoin seed with the oil extracted (hereafter referred to as extracted sainfoin seed); 3) autoclaved sainfoin seed; 4) extracted, autoclaved sainfoin seed; 5) SBM; and 6) autoclaved raw soybean meal which had the oil extracted. The oil was extracted from protein sources 2, 4 and 6 by soaking the seed in hexane for 7 days, pouring off the liquid, and rinsing the seed in warm water to remove any residual hexane remaining. Protein sources 3, 4 and 6 were heated in an autoclave for two hours at 107C.

Proximate analyses, calcium and phosphorous determinations were made on each of the protein sources used, and on the diets fed (1). Percentage composition of the diets fed are in Table 25. Amino acid analyses (57) and trypsin inhibitor determinations (26) were performed on the protein sources fed.

Diet treatments were randomly assigned to rats which had been stratified for initial weight and cage level. Each rat was caged individually in standard racks in a temperature controlled room, with water and feed supplied *ad libitum*. Feeders were equipped with

Table 25. Percentage Composition of Sainfoin and Soybean Meal Diets Fed to Rats. Trial II.

Ingredients	Protein Source					
	Sainfoin	Sainfoin extracted	Sainfoin autoclaved	Sainfoin autoclaved extracted	SBM	Raw soybeans autoclaved extracted
Sainfoin	56.16	---	---	---	---	---
Sainfoin, extracted	---	53.06	---	---	---	---
Sainfoin, autoclaved	---	---	56.59	---	---	---
Sainfoin, autoclaved extracted	---	---	---	53.59	---	---
Soybean meal	---	---	---	---	44.06	---
Raw soybeans, autoclaved extracted	---	---	---	---	---	43.09
Corn starch	37.66	38.82	38.28	38.31	48.72	49.09
Corn oil	1.59	3.54	0.53	3.58	3.10	3.56
Calcium carbonate	1.77	1.76	1.77	1.75	1.66	1.64
Monosodium phosphate	1.57	1.57	1.58	1.52	1.21	1.37
Vitamin trace mineral mix[1]	0.75	0.75	0.75	0.75	0.75	0.75
Salt	0.50	0.50	0.50	0.50	0.50	0.50

1 - Vitamin A - 500,000 USP units, Vitamin D_3 - 100,000 IC units, Vitamin E - 1,000 I units, Vitamin B_{12} - 4 mg, Riboflavin - 700mg, Niacin - 4,000 mg, d-Pantothenic Acid - 2,000 mg. choline - 100,000 mg, Zinc - 9,100 mg, Iron - 4,500 mg, Manganese - 2,500 mg, Copper - 450 mg, Cobalt - 45 mg, Iodine - 68 mg per 454 g of vitamin-mineral mix.

partial covers and placed in ceramic dishes to minimize wastage. Feed consumption and body weight for individual rats were recorded every 7 days and at termination after 28 days. Five rats from each diet were then fasted for 18 hours, sacrificed, bled and the pancreases removed and weighed to the nearest fourth decimal. Total gain, feed consumption, feed efficiency and protein efficiency ratios were computed for individual rats and were analyzed by least squares analysis with initial weight as covariate (21). Analysis of covariance, adjusting for fasted weight, was used to detect differences in pancreas size. Individual mean differences were distinguished with Duncan's New Multiple Range Test (12) where significance ($P<.05$) occurred.

Trial III

Six isonitrogenous, isocaloric diets containing 11% protein and 4% oil were fed to 60 female rats of the Holtzman strain (Table 26). The average initial weight of the rats fed each diet was approximately 72.5 g. The protein sources for these rations were: 1) sainfoin seed; 2) extracted sainfoin seed; 3) sainfoin seed which had been autoclaved at 107C for one hour; 4) sainfoin seed which had been autoclaved at 107C for two hours; 5) casein; and 6) SBM.

The materials and methods used in this trial were as described for Trial II.

Table 26. Percentage Composition of Sainfoin, Soybean Meal and Casein Diets Fed to Rats. Trial III.

Ingredients	Protein Source: Sainfoin	Sainfoin extracted	Sainfoin autoclaved 1 hr	Sainfoin autoclaved 2 hr	Casein	SBM
Sainfoin	29.15	---	---	---	---	---
Sainfoin, extracted	---	30.63	---	---	---	---
Sainfoin, autoclaved 1 hr	---	---	29.16	---	---	---
Sainfoin, autoclaved 2 hr	---	---	---	30.27	---	---
Casein	---	---	---	---	12.17	---
Soybean meal	---	---	---	---	---	22.78
Corn starch	60.28	57.97	61.00	60.04	71.88	63.74
Corn oil	2.09	3.01	1.30	1.27	3.97	3.69
Alphacel[1]	2.26	2.13	2.26	2.16	5.00	3.64
Calcium carbonate	0.69	0.69	0.70	0.69	0.80	0.63
Monosodium phosphate	1.28	1.32	1.33	1.32	1.93	1.27
Mineral mix[2]	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix[3]	2.20	2.20	2.20	2.20	2.20	2.20

1 - Nutritional Biochemical Company, non-nutritive cellulose.

2 - Salt Mixture, USP XIV.

3 - Nutritional Biochemical Company, Vitamin diet fortification mixture.

RESULTS AND DISCUSSION EXPERIMENT II

Trial I

Chemical analyses of the sainfoin and SBM protein sources fed to weanling pigs agreed with previous findings (25,65) that sainfoin seed has less protein, more oil and less ash than commercial SBM (Table 27). Although sainfoin seed has less total protein than SBM, the two protein sources are quite similar in essential amino acid composition as determined by amino acid analyses (Table 28). Both protein sources should satisfy the essential amino acid requirements of weanling pigs (46).

Table 27. Chemical Analyses of Protein Sources Fed to Weanling Pigs. Trial I. (%)

Protein Source	H_2O	Protein	Ether extract	Ash	Calcium	Phosphorous
Soybean meal	7.1	50.4	1.5	6.3	0.38	0.72
Sainfoin	6.8	35.9	4.9	4.4	0.17	0.35
Extracted sainfoin	9.8	36.5	3.1	4.2	0.19	0.37
Wheat	8.8	11.1	1.0	1.9	0.08	0.38

The oil content of sainfoin seed and SBM were 4.9% and 1.5%, respectively (Table 27). The low oil content of SBM was due to solvent extraction in commercial processing which removes most of the oil. The extracted sainfoin seed had 3.1% oil compared to 4.9% oil

Table 28. Amino Acid Analyses of Sainfoin Seed, With and Without Oil, and Commercially Prepared Soybean Meal.[1] Trial I.

	A. A. Content Expressed as a % of Seed Wt.			A. A. Content Expressed as a % of Total Protein		
Amino Acid	Sainfoin	Sainfoin extracted	Soybean	Sainfoin	Sainfoin extracted	Soybean
Lysine[2]	2.15	1.74	2.55	6.12	6.19	6.10
Histidine[2]	1.54	1.24	0.96	4.39	4.41	2.30
Ammonia	0.71	0.61	0.82	2.02	2.17	1.96
Arginine[2]	3.91	3.01	3.10	11.14	10.70	7.42
Aspartic Acid	3.83	3.07	5.10	10.91	10.92	12.20
Threonine[2]	1.24	0.98	1.66	3.54	3.49	3.97
Serine	1.65	1.33	2.22	4.70	4.73	5.31
Glutamic Acid	7.39	5.90	8.96	21.05	20.98	21.44
Proline	1.58	1.26	2.18	4.50	4.48	5.22
Glycine	1.56	1.28	1.58	4.44	4.55	3.78
Alanine	1.26	1.00	1.74	3.59	3.56	4.16
Half Cystine	--	--	--	--	--	--
Valine[2]	1.56	1.23	1.98	4.44	4.37	4.74
Methionine[2]	0.59	0.54	0.62	1.68	1.92	1.48
Isoleucine[2]	1.37	1.07	1.88	3.90	3.81	4.50
Leucine[2]	2.40	1.90	2.34	6.84	6.76	5.60
Tyrosine	0.99	0.84	1.64	2.82	2.99	3.92
Phenylalanine[2]	1.38	1.12	2.46	3.94	3.98	5.89
Total	35.11	28.12	41.79	100.02	100.01	99.99
% Kjeldahl Prot. (N x 6.25)	35.9	36.5	50.35			

1 - Tryptophane was destroyed, only a trace of cystine was recovered and the methionine recovery was possibly reduced due to the acid hydrolysis and presence of excess carbohydrate.

2 - Essential amino acid.

for the non-extracted seed, indicating the oil extraction process was inefficient. It was hypothesized the hexane removed most of the oil from the seed, but that a high percentage of this oil remained on the surface of the seed as a residue. The hypothesis was later confirmed by adding a warm water rinse to the extraction procedure. This modification was incorporated in seed preparation methods for Trials II and III.

The diets fed to the weanling pigs were quite similar, except for ether extract content (Table 29). The diet with sainfoin seed as the protein supplement had the highest oil content with 2.3%. Those diets with SBM plus sainfoin oil, and extracted sainfoin seed as protein supplements had 1.8% and 1.7%, respectively, and the diet with SBM as the protein supplement had the lowest oil content, 1.1%.

Table 29. Chemical Analyses of Soybean Meal and Sainfoin Diets Fed to Weanling Pigs. Trial I. (%)

Ration	H_2O	Protein	Ether extract	Ash	Calcium	Phosphorous
Soybean meal	7.9	20.2	1.1	6.8	.78	.71
Soybean meal + sainfoin oil	7.9	19.8	1.8	6.9	.82	.68
Sainfoin	7.7	20.3	2.3	6.7	.81	.69
Extracted sainfoin	7.9	19.9	1.7	6.6	.79	.70

Comparisons of sainfoin seed and SBM, with and without sainfoin oil, as protein supplements for weanling pigs are in Table 30. Significant differences between rations were not detected for average daily gain, total gain, daily feed consumed and feed per gain ratios, although the data suggested the pigs on the SBM diets performed better than the pigs on the sainfoin diets.

Table 30. Comparison of Sainfoin Seed and Soybean Meal, With and Without Sainfoin Oil, as Protein Supplements in the Diets of Weanling Pigs. Trial I.

Measurement	SBM	SBM + Sainfoin oil	Sainfoin seed	Extracted sainfoin seed
No. animals	12	12	12	12
Avg initial wt., kg	14.2	14.3	14.4	14.4
Avg final wt., kg	23.7	23.2	21.2	21.3
Avg total gain, kg	9.5	8.9	6.8	6.9
Avg daily gain, kg	0.56	0.52	0.40	0.40
Avg daily feed, kg	1.24	1.10	1.08	1.10
Avg feed/gain ratio	2.21	2.11	2.70	2.75

Comparisons between diets with SBM and sainfoin seed as protein supplements, disregarding whether or not they had sainfoin oil (Table 31), and between diets with and without sainfoin oil, disregarding the protein source (Table 32) were made. These data indicated that pigs on the SBM diets had significantly higher average daily gains, average total gains, and converted their feed to weight significantly better than the pigs on the sainfoin diets (Table 31). Sainfoin oil did not significantly affect average daily gains, average total gains, feed consumption or feed per gain ratios (Table 32).

Table 31. Comparisons of Sainfoin Seed with Soybean Meal as Protein Supplements in Diets of Weanling Pigs. Trial I.

Measurement	SBM	Sainfoin seed
No. animals	24	24
Avg initial wt., kg	14.3	14.4
Avg final wt., kg	23.5	21.2
Avg total gain, kg	9.2 a[1]	6.8 b
Avg daily gain, kg	0.54 a	0.40 b
Avg daily feed, kg	1.17	1.09
Avg feed/gain ratio	2.17 b	2.74 a

1 - Means in the same line followed by different letters differ significantly (P<.05).

Lower feed consumption of the diets with the higher oil contents was expected because fats and oils furnish approximately 2.25 times as much energy as do carbohydrates, and animals tend to consume feed until they have met their energy requirements. Feed consumption data indicated this was the case, although differences in feed intake were not statistically different (Tables 30 and 32).

Table 32. Effect of Sainfoin Oil in the Diet of Weanling Pigs. Trial I.

Measurement	With sainfoin oil	Without sainfoin oil
No. animals	24	24
Avg initial wt., kg	14.4	14.3
Avg final wt., kg	22.2	22.5
Avg total gain, kg	7.9	8.2
Avg daily gain, kg	0.46	0.48
Avg daily feed, kg	1.08	1.17
Avg feed/gain ratio	2.35	2.43

These data indicate pigs perform better on diets supplemented with SBM than on diets supplemented with sainfoin seed, although the amino acid analyses of the two protein supplements suggest they should have equal feeding values. It is felt the duration of the trial was too short to accurately evaluate sainfoin seed as a protein supplement for swine. Another feeding trial in which weanling pigs are fed until they reach market weight should be conducted.

Trial II

The objectives of Trial II were to: 1) compare the protein quality of sainfoin seed with SBM; 2) determine the effect, if any, of sainfoin oil in the diet; and 3) determine if the trypsin inhibitor in sainfoin seed has a detrimental effect on feeding value. Raw soybean meal, which had the oil extracted and had been autoclaved, was included as a source of protein to determine if the processing of the protein sources had a detrimental effect on their feeding value.

The extracted autoclaved soybean protein source was quite similar in chemical analyses to commercially prepared SBM (Table 33). Although the sainfoin seed used for the four sainfoin protein sources came from the same seed source, the two sainfoin protein sources, which had been extracted, had more crude protein than the two sainfoin protein sources which had not been extracted, due to removal of the oil. Ether extract determinations indicated the autoclaved sainfoin seed had 6.14% oil compared to 4.29% for the unprocessed sainfoin seed. The higher oil content of autoclaved seed may have been due to the heat hydrolyzing glycolipids, phospholipids and other complex lipid compounds which would not have been readily extracted by ether alone (10).

Amino acid analyses of the six protein sources indicated again that sainfoin seed is quite similar in essential amino acid composition to SBM (Table 34). Processing of sainfoin and soybean seed did not appear to appreciably affect their amino acid composition. All six protein sources should meet the essential amino acid requirements of weanling rats (46).

Table 33. Chemical Analyses of Protein Sources Fed to Weanling Rats. Trial II. (%)

Protein Source	H_2O	Protein	Ether extract	Ash	Calcium	Phosphorous
Sainfoin	6.77	35.61	4.29	4.41	0.16	0.40
Sainfoin, extracted	9.23	37.69	0.86	3.92	0.18	0.42
Sainfoin, autoclaved	6.63	35.34	6.14	4.25	0.16	0.39
Sainfoin, autoclaved extracted	7.72	37.32	0.78	3.63	0.19	0.44
Soybean meal	7.11	45.39	2.04	6.43	0.31	0.70
Raw soybeans, autoclaved extracted	9.62	46.41	1.02	6.52	0.34	0.63

The assay used to detect trypsin inhibitor activity in the protein sources does not quantify the amount of inhibitor present, but gives a relative measure of the percent inhibition in relation to a standard which does not contain any inhibitor (26). This assay indicated sainfoin seed was high in inhibitory activity with 62% inhibition of the enzyme (Table 35). Extracting the oil from sainfoin seed reduced the activity to 10% inhibition. Autoclaving the sainfoin seed reduced the activity to 8%. The autoclaved extracted sainfoin seed, SBM and autoclaved extracted soybean protein sources did not have any inhibitory activity. These data suggested the inhibitor may be contained in the lipoprotein fraction of the seed, or denatured by autoclaving the seed. It was hypothesized the rats fed raw sainfoin would

Table 34. Amino Acid Composition of Protein Sources Fed to Weanling Rats Expressed as a Percentage of Protein Recovered.[1] Trial II.

Amino Acid	Sainfoin	Sainfoin extracted	Sainfoin autoclaved	Sainfoin extracted autoclaved	SBM	Soybeans extracted autoclaved
Lysine[2]	6.12	5.44	5.47	5.47	6.32	5.49
Histidine[2]	4.49	3.96	3.96	3.86	2.57	2.43
Arginine[2]	12.10	11.70	12.00	11.61	7.18	7.34
Aspartic Acid	10.88	10.98	11.75	11.09	11.82	11.87
Threonine[2]	3.39	3.46	3.37	3.55	3.89	3.91
Serine	4.87	5.10	4.76	5.10	5.01	5.07
Glutamic Acid	21.43	21.55	21.71	21.58	20.98	21.01
Proline	4.10	4.15	4.11	4.23	4.58	4.61
Glycine	3.98	4.15	3.99	4.26	3.68	3.74
Alanine	3.48	3.52	3.46	3.58	3.98	4.00
Half Cystine	1.25	1.10	1.21	1.05	2.02	1.86
Valine[2]	4.04	4.22	4.05	4.26	4.62	4.55
Methionine[2]	1.25	1.29	1.11	1.27	1.16	1.22
Isoleucine[2]	3.54	3.71	3.83	3.74	4.41	4.37
Leucine	6.69	6.98	6.59	6.98	7.87	7.82
Tyrosine	2.73	2.74	3.00	2.59	3.56	3.43
Phenylalanine[2]	3.89	4.06	3.77	4.01	4.32	5.31
Ammonia	1.78	1.89	1.86	1.76	2.04	1.97

1 - Tryptophan was destroyed and cystine and methionine recovery was possibly reduced due to acid hydrolysis and presence of excess carbohydrate.

2 - Essential Amino Acid.

not perform as well as rats fed the other sources of protein, and that they would have enlarged pancreases.

Table 35. Trypsin Inhibitor Assay of the Protein Sources Fed to Weanling Rats. Trial II.

Protein Source	Percent Inhibition
Sainfoin	62
Sainfoin, extracted	10
Sainfoin, autoclaved	8
Sainfoin, autoclaved extracted	0
Soybean meal	0
Raw soybeans, autoclaved extracted	0

During the course of the feeding trial, several rats became ill, refused food and two rats died. An autopsy of one ill rat and one dead rat, conducted by the Animal Health Division, Diagnostic Laboratory at Bozeman, revealed the rats had pneumonia caused by the organism Proteus mirabulis. It was postulated the rats contracted the disease during shipping, and their illness was not a result of the feeding trial. A total of twelve rats displaying symptoms of pneumonia were removed from the trial.

Significant differences between diets were not detected for average daily gain and protein efficiency ratios (Table 36). Rats fed the autoclaved extracted soybean diet consumed less feed ($P<.05$)

Table 36. Performance of Rats Fed Sainfoin and Soybean Meal as Protein Sources in the Diet.[1] Trial II.

Measurement	Sainfoin	Sainfoin extracted	Sainfoin autoclaved	Sainfoin autoclaved extracted	SBM	Soybeans autoclaved extracted
No. animals	13	13	14	14	13	11
Avg initial wt., g	69.0	69.4	68.3	68.8	68.8	69.7
Avg gain, g	97.0	90.7	101.6	95.1	100.4	87.6
Avg final wt., g	166.0	160.1	169.9	163.9	169.2	157.3
Avg daily gain, g	3.56	3.34	3.71	3.43	3.67	3.02
Avg daily feed, g	13.42 a[3]	13.54 a	13.98 a	14.11 a	13.08 ab	12.16 b
Avg feed/gain ratio	3.82 ab	4.10 a	3.77 ab	4.12 a	3.58 b	4.08 a
Avg PER[2]	1.31	1.27	1.30	1.22	1.37	1.26

1 - Least square means.

2 - Protein efficiency ratio.

3 - Means in the same line followed by different letters differ significantly (P<.05).

than did rats fed the four sainfoin diets. Significant differences in feed consumption between SBM and the four sainfoin diets were not detected. Rats fed SBM had better feed efficiencies (P<.05) than rats fed extracted sainfoin, autoclaved extracted sainfoin and autoclaved extracted soybeans. There were no differences in feed efficiencies of the rats fed the four sainfoin diets, or between those fed the four sainfoin diets and autoclaved extracted soybeans. Rats fed sainfoin and autoclaved sainfoin utilized their feed as efficiently as rats fed SBM.

These data indicated there were no differences in food value for weanling rats between the four sainfoin diets, and that they were equal to SBM in terms of average daily gain, average daily feed consumed and protein efficiency ratios. The autoclaved extracted soybean diet appeared to have the poorest feeding value. It is possible that autoclaving the soybeans at 107C for two hours had a detrimental effect, although it did not appear to affect the feeding value of sainfoin seed (23).

Pancreas weights of a random sample of five rats fed each diet indicated the trypsin inhibitor in sainfoin did not enlarge the pancreases (Table 37). Significant differences in pancreas weight were not detected between rats fed sainfoin, autoclaved sainfoin, SBM or autoclaved extracted soybean. Rats fed extracted sainfoin and autoclaved extracted sainfoin diets had larger pancreases ($P<.05$) than did rats fed raw sainfoin. No explanation may be given for this effect. These data indicated the trypsin inhibitor in sainfoin does not cause hypertrophy of the pancreas or decrease the feed value as is the case when raw soybeans are fed.

Table 37. Pancreas Weights of Rats Fed Sainfoin and Soybean Meal as Protein Sources in the Diet. Trial II.

Ration	Pancreas weight, g
Sainfoin	.7599 b[1]
Sainfoin, extracted	.9188 a
Sainfoin, autoclaved	.8092 ab
Sainfoin, autoclaved extracted	.9516 a
Soybean meal	.8022 ab
Soybean, autoclaved extracted	.8111 ab

1 - Means in the same column followed by different letters differ significantly ($P<.05$).

Trial III

The purpose of Trial III was to substantiate the findings of Trial II, and to determine if autoclaving sainfoin for two hours at 107 C had a detrimental effect on its feeding value. A casein diet was included in this trial as a control.

Extracted sainfoin had less crude protein than sainfoin (Table 38). The lower crude protein content of extracted sainfoin was due to its high moisture content. Crude protein on a dry weight basis for sainfoin and extracted sainfoin was 37.7% and 38.9%, respectively. Ether extract determinations again revealed that autoclaved sainfoin seed had more oil than sainfoin seed which had not been autoclaved. Extracted sainfoin had 3.26% oil as compared to 0.86% oil in Trial II. The procedures used to remove the oil were the same in both trials, and the reason for the discrepancies in oil content in Trials II and III is not known.

Table 38. Chemical Analyses of the Protein Sources Fed to Weanling Rats. Trial III. (%)

Protein Source	H_2O	Protein	Ether extract	Ash	Calcium	Phosphorous
Sainfoin	4.37	36.03	6.57	3.76	0.16	0.50
Sainfoin, extracted	11.82	34.29	3.26	3.11	0.15	0.45
Sainfoin, autoclaved 1 hr	4.95	36.01	9.28	3.86	0.14	0.46
Sainfoin, autoclaved 2 hr	7.98	34.69	9.02	3.75	0.15	0.45
Casein	7.46	86.27	0.26	--	--	--
Soybean meal	9.00	46.11	1.38	6.30	0.50	0.76

Amino acid analyses of the six protein sources are in close agreement with those found in Trials I and II, and substantiate the similar essential amino acid composition of sainfoin and SBM (Table 39).

Table 39. Amino Acid Composition of Protein Sources Fed to Weanling Rats in Percentage of Protein Recovered.[1] Trial III.

Amino Acid	Sainfoin	Sainfoin extracted	Sainfoin autoclaved 1 hr	Sainfoin autoclaved 2 hr	Casein[2]	SBM
Lysine [3]	6.35	6.06	6.02	5.76	7.74	6.52
Histidine [3]	4.16	3.98	3.93	3.85	2.77	2.47
Arginine [3]	11.76	11.75	11.50	11.27	3.76	7.45
Asparatic Acid	11.08	10.83	11.18	11.45	6.64	11.80
Threonine [3]	3.62	3.83	3.64	3.82	4.20	3.87
Serine	5.14	5.43	5.13	5.18	5.75	4.76
Glutamic Acid	20.82	20.46	21.01	21.13	21.46	20.54
Proline	4.29	4.69	4.42	4.36	10.29	5.03
Glycine	4.26	4.58	4.28	4.39	1.66	3.80
Alanine	3.62	3.83	3.64	3.67	2.54	4.11
Half Cystine	--	--	--	--	.55	--
Valine [3]	3.89	4.28	3.92	3.96	7.52	4.77
Methionine [3]	1.04	1.30	1.49	1.80	2.99	1.04
Isoleucine [3]	3.45	3.75	3.50	3.63	6.31	4.60
Leucine [3]	6.56	6.99	6.62	6.84	9.51	7.77
Tyrosine	2.91	2.90	2.90	2.77	5.20	3.48
Phenylalanine [3]	3.95	4.20	3.93	3.99	5.09	5.22
Ammonia	1.99	2.26	2.09	2.12	--	2.56

1 - Tryptophan was destroyed, only a trace of cystine was recovered and the methionine recovery was possibly reduced due to acid hydrolysis and presence of excess carbohydrate.

2 - Data obtained from the Atlas of Nutritional Data on United States and Canadian Feeds

3 - Essential Amino Acid.

The trypsin inhibitor assay indicated raw sainfoin had the highest inhibitory activity with 51% inhibition (Table 40). The inhibitory activity in extracted sainfoin and one hour autoclaved sainfoin was 44% and 15%, respectively. Sainfoin which had been autoclaved for two hours, casein and SBM did not exhibit any inhibitory activity. These data indicate that heating raw sainfoin greatly reduces or eliminates the activity of the inhibitor in raw sainfoin seed.

Table 40. Trypsin Inhibitor Assay of the Protein Sources Fed to Weanling Rats. Trial III.

Protein Source	Percent Inhibition
Sainfoin	51
Sainfoin, extracted	44
Sainfoin, autoclaved 1 hr	15
Sainfoin, autoclaved 2 hr	0
Casein	0
Soybean meal	0

During the course of the trial, eight rats contracted pneumonia and were removed from the experiment. Rats fed sainfoin seed as a protein source gained as well as rats fed casein and SBM, utilized their feed as efficiently as rats on SBM, but did not utilize their protein as efficiently as rats fed either casein or SBM (Table 41). Rats fed the two autoclaved sainfoin diets gained as well as rats fed sainfoin, but did not utilize the feed as efficiently. The performance of rats fed extracted sainfoin was the poorest of any of the rations.

These data indicate it is not necessary to heat or extract the oil from sainfoin seed to obtain maximum feeding value. Processing of the seed, particularly removing the oil, appears to have a detrimental effect.

There were no differences in pancreas weight due to diets (Table 42). Performance and pancreas data indicate the inhibitor in sainfoin does not decrease the feeding value or result in hypertrophy of the pancreas, as is the case when raw soybeans are fed (Tables 41 and 42).

Table 41. Performance of Rats Fed Sainfoin Seed, Soybean Meal and Casein as Protein Sources in the Diets.[1] Trial III.

Measurement	Sainfoin	Sainfoin extracted	Sainfoin autoclaved 1 hr	Sainfoin autoclaved 2 hr	Casein	SBM
No. animals	8	8	8	10	9	9
Avg initial wt., g	72.5	73.5	72.6	72.2	72.3	72.0
Avg gain, g	89.7	77.6	82.8	84.8	90.8	96.0
Avg final wt., g	162.2	151.1	155.4	156.4	163.1	168.0
Avg daily gain, g	3.28 ab[3]	2.80 c	3.04 bc	3.04 bc	3.32 ab	3.44 a
Avg daily feed, g	14.74	14.59	14.47	14.14	13.29	14.61
Avg feed/gain ratio	4.48 bc	5.22 e	4.76 d	4.69 d	4.00 a	4.26 b
Avg PER[2]	1.94 c	1.68 d	1.90 cd	1.89 cd	2.24 a	2.09 b

1 - Least square means.

2 - Protein efficiency ratio.

3 - Means in the same line followed by different letters differ significantly.

Table 42. Pancreas Weights of Rats Fed Sainfoin, Soybean Meal and Casein as Protein Sources in the Diets. Trial III.

Ration	Pancreas Weight, g
Sainfoin	.7542
Sainfoin, extracted	.6411
Sainfoin, autoclaved 1 hr	.6706
Sainfoin, autoclaved 2 hr	.7144
Casein	.9173
Soybean meal	.8083

SUMMARY AND CONCLUSIONS EXPERIMENT II

One swine and two rat feeding trials were conducted to evaluate sainfoin seed as a source of protein for monogastric animals. Chemical and amino acid analyses revealed sainfoin has approximately 36% crude protein, and its essential amino acid composition is quite similar to SBM. Performance data indicated weanling pigs performed better on SBM than on sainfoin seed; however, weanling rats on 20% protein diets performed equally well on sainfoin and SBM. When the protein content of the diets was restricted to 11% protein, weanling rats on sainfoin gained as well as rats fed SBM and casein, utilized their feed as well as rats fed SBM, however they did not utilize the protein quite as efficiently as rats fed SBM or casein. The rat data indicated the protein in sainfoin is of high quality and shows promise as a protein supplement. The duration of the weanling pig trial may have been too short to accurately evaluate sainfoin seed as a protein supplement for swine. A feeding trial with weanling pigs fed to market weight should be conducted.

Significant differences between animals fed sainfoin and extracted sainfoin diets were not detected in Trials I and II. In Trial III, rats fed extracted sainfoin seed did not perform as well as rats fed sainfoin in which the oil had not been removed. These data indicate it is not necessary to remove the oil from sainfoin seed to obtain maximum feeding value, and in some cases removal of the oil may be detrimental.

The assay for trypsin inhibitor revealed that raw sainfoin was high in inhibitory activity, and that this activity was drastically reduced or nullified when the seed was autoclaved. Performance and pancreas data, however, indicated the inhibitor did not have a detrimental effect on the feed value of sainfoin, and the pancreases from rats fed raw sainfoin were not enlarged. Thus, it does not appear to be necessary to inactivate the inhibitor to obtain maximum feed value, as is the case with soybeans. The amount of inhibitor in the seed is not known. It is altogether possible there is not enough inhibitor present to be detrimental, or that it is destroyed in the gastric region of the intestines.

These data indicate sainfoin seed shows promise as a protein supplement, and its possible use for this purpose should be further investigated.

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