extraction of solids or the extraction of nitrogen. A grinding process which gives a high degree of rupture of cells appears to be important in obtaining good yields. Maintaining a low temperature is especially important in obtaining efficient extraction of the nitrogenous constituents.

Grinding methods which do not give a high degree of rupture of cells while maintaining a low temperature, such as the large-scale laboratory process studied here, apparently cannot be expected to give a good yield of extractable material. The studies reported here on methods of expressing the juice from the ground mass are too limited to allow for concise conclusions, but it appears that the small samples and the manner of the squeezing were both compatible to good yields.

The processing of freshly harvested alfalfa for a good yield of material, high in protein, would appear possible through the use of grinding, pressing, and heat treatment similar to what is described here. A residue having some apparent value as a roughage feed is also obtained. However, a third fraction in liquid form, which contains much of the nutritive value of the alfalfa, results from the processing. While the heat-precipitated material might be easily dried and the residue might be stored and fed as silage, a concentration of the liquid fraction would be costly and difficult. Nevertheless, when processing grasses for their protein is considered, the methods discussed here appear more suitable than alkali extraction methods or methods not involving the addition of water. Feeding studies on the various fractions are essential, however, to a more thorough evaluation of the methods.

Because the data obtained from extracting the alfalfa could be closely duplicated with similar or different samples, little attention was given to factors such as moisture content of the alfalfa, during the work. Samples which contained between 63 and 72% of moisture showed no relationship between moisture content and extractability of solids or nitrogen when the collections were made between late August and the middle of October. Further, no changes attributable to advancement of the season were evident during the same period. Data recorded with a sample collected in June (Table III) showed some divergence from those obtained with samples collected in September (Table I) in that a lower percentage of extractable solids was obtained with the sample collected at the earlier time. No differences of nitrogen extractability were apparent between the samples collected in June and September, however. Thus, it seems evident that variables such as the season of harvest and the stage of maturity should receive additional attention in alfalfa extraction work.

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ALFALFA CONCENTRATES IN NUTRITION

Protein Quality of an Alfalfa Concentrate

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THE USE OF FRESH HERBAGE as a raw material for the preparation of protein concentrates has received attention in recent years (*12*). Some investigators have processed plants such as alfalfa and Italian ryegrass to obtain the juice, a liquid suspension containing soluble protein, nonprotein matter and chloroplasts. The juice upon treatment with steam yielded a heat-precipitable material of high nitrogen and low fiber content. This material was suitable for use in high energy diets in either wet or dry form (*3, 9, 13*).

Feeding studies with the heat-precipitable material have indicated that it is a fair to moderately good source of protein in practical diets. Work with chicks showed that diets containing the material in dry form supported rather good growth although the rate of growth was somewhat inferior to that obtained with a diet supplemented with casein (*3*). The diets containing the alfalfa preparations were improved by cholesterol supplementation and those containing either alfalfa or other herbage preparations were improved by lysine supplementation. Experiments using a wet preparation from alfalfa for chicks suggested that the material was equal to or slightly inferior to fish meal (*9*). In work using a dried concentrate from alfalfa for laying hens, egg production was similar for diets that contained either an alfalfa concentrate or fish meal (*9*).

The work reported here involved rat feeding experiments with an alfalfa concentrate, an alfalfa meal, and some supplements as dietary nitrogen sources. Cholesterol, a compound known to counteract largely the growth inhibitory property of alfalfa meal (*11*), was also used as a supplement.

Experimental

The alfalfa concentrate was prepared by processing several varieties of fresh alfalfa at the prebloom and early bloom stages. Following the preparation of the dry concentrate by a large scale process as described in a previous report (*6*), the material was stored in glass bottles at room temperature prior to feeding, the storage intervals being about 2 months for Experiment 1 and 18 months for Experiments 2 and 3. The material, ground through a 1-mm. sieve with the Wiley mill, was analyzed (*1*) and found to contain about 50% protein (N × 6.25) and 1% crude fiber.

The protein sources used in the diets had the following nitrogen contents as determined by the Kjeldahl method (*1*):

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Rat experiments on the evaluation of a protein concentrate prepared from freshly harvested alfalfa are reported. Growth, feed efficiency, and protein efficiency measurements were made with diets containing the concentrate and/or other protein or amino acids as the nitrogen source. The protein of the concentrate was of low quality as shown by the responses obtained by supplementing with other nitrogen sources such as methionine plus lysine, fish meal, soybean protein, or soybean protein plus methionine. Of the two amino acids observed to be deficient, methionine was more limiting than lysine. The addition of cholesterol to diets containing the concentrate resulted in small improvements in growth and feed utilization, especially when another protein source was not present in the diet. The use of alfalfa meal as a nitrogen source in somewhat similar diets gave comparable responses except that a lysine deficiency was not demonstrated.

alfalfa concentrate, 8.45% (Experiment 1), 7.80% (Experiments 2 and 3); alfalfa meal, 3.27%; soybean protein, 13.55%; fish meal, 9.84%; gluten, 13.43%; casein, 14.01%. The alfalfa meal was a batch of field-cured alfalfa which was ground in the Wiley mill using a 1-mm. sieve.

Diets which contained the different protein sources were fed to young rats, and growth and feed utilization were measured in several experiments. Experiment 1 involved a comparison of a diet containing the alfalfa concentrate with similar diets containing casein, soybean protein (Drackett Co.), and gluten as single nitrogen sources. Experiment 2 involved the effect of supplementing a diet containing the alfalfa concentrate with other nitrogen sources such as the amino acids, methionine and lysine, and the protein sources, soybean protein or fish meal. An alfalfa meal was also used in Experiment 2. Experiment 3 involved the effect of cholesterol supplementation of the alfalfa concentrate or meal diets. Comparisons were generally made at equivalent nitrogen levels—i.e., 1.6 and 3.2% of nitrogen in the diets.

The experiments were of 18 or 21 days duration. Young albino rats of the Sprague-Dawley strain were used. Male animals were used in Experiment 1 and females in Experiments 2 and 3. Initial average weights per animal were 62, 53, and 47 grams in Experiments 1, 2, and 3, respectively. Five animals constituted a group in each experiment. The animals were housed in individual wire mesh cages and were given food and water *ad libitum*.

Individual weights were obtained weekly. Feed intake was measured on a daily and individual basis in Experiment 1 and on a periodic and subgroup basis in Experiments 2 and 3. Feed and protein efficiencies were calculated from the total gain and feed consumption of an entire group. As used here, feed efficiency represents the grams of gain in body weight per gram of diet consumed, and protein efficiency represents the grams of gain per gram of dietary nitrogen consumed.

Table I. Growth and Feed Utilization Responses Obtained with Alfalfa Concentrate and Other Single Protein Sources[a,b]

Nitrogen Source in Diets	Nitrogen Content of Diets, %	Gain/Rat, Grams	Feed Efficiency[c]	Protein Efficiency[c]
Alfalfa concentrate	1.6	3	0.09	5.3
Gluten	1.6	2	0.03	1.9
Casein	1.6	78	0.29	17.9
Soybean protein	1.6	51	0.22	14.0
Alfalfa concentrate	3.2	48	0.30	9.3
Gluten	3.2	23	0.22	6.7
Casein	3.2	146	0.44	13.8
Soybean protein	3.2	112	0.39	12.1

[a] 21-Day experiment.
[b] Diets not supplemented with cholesterol.
[c] Values calculated from feed intake measurements taken during the 10 to 21 days of the experiment. Feed efficiency values represent grams of gain in body weight per gram of diet consumed, and protein efficiency values represent grams of gain per gram of dietary nitrogen consumed.

Table II. Effects of Supplementing Alfalfa Diets with Other Nitrogen Sources[a,b]

Nitrogen Sources in Diets						
Alfalfa protein, 1.6% N in diet per source	Other protein, 1.6% N in diet per source	Amino Acids[c]	Nitrogen Content of Diets, %	Gain/Rat, Grams	Feed Efficiency[d]	Protein Efficiency[d]
Concentrate			1.60	4	0.04	2.2
Concentrate		Methionine	1.65	29	0.18	11.1
Concentrate		Methionine and lysine	1.77	49	0.30	17.0
Concentrate	Soybean protein		3.20	63	0.34	10.6
Concentrate	Soybean protein	Methionine	3.24	85	0.46	14.4
Concentrate	Fish meal		3.20	56	0.31	9.7
Meal			1.60	10	0.10	6.4
Meal		Methionine	1.65	32	0.21	12.4
Meal		Methionine and lysine	1.77	34	0.25	13.9
Meal	Soybean protein		3.20	71	0.39	12.1
Meal	Soybean protein	Methionine	3.24	88	0.49	15.1
Meal	Fish meal		3.20	68	0.36	11.2
	Gluten		1.60	−1	. . .	. . .
	Casein		1.60	66	0.34	21.2
	Soybean protein		1.60	41	0.25	15.9
	Fish meal		1.60	24	0.16	10.0
	Fish meal and soybean protein		3.20	82	0.43	13.3

[a] 18-Day experiment.
[b] Alfalfa diets contained 1% of cholesterol.
[c] Methionine was added at a level of 0.5% in diets with alfalfa nitrogen as the only protein source and at a level of 0.4% when alfalfa nitrogen and soybean nitrogen were both present as protein sources; lysine was added at a level of 0.8%.
[d] See footnote, Table I; values determined from data of the entire experimental period.

The diet consisted of the following, in percentages: corn starch plus a protein source, 85.56; Crisco, 10.00; USP salt mixture XIV, 4.00; sodium sulfate, 0.20; choline chloride, 0.10; vitamins, 0.14. When alfalfa meal was used as a protein source, the fat content of the diet was increased to 30% to compensate for the presence of this high fiber ingredient in the diet. The adjustment of the

Table III. Changes in Responses Obtained from the Presence of Cholesterol in Alfalfa Diets[a]

Nitrogen Sources in Diets			Changes in Responses[b]		
Alfalfa protein, 1.6 or 3.2% N in diet per source	Other protein, 1.6% N in diet per source	Nitrogen Content of Diets, %	Gain/rat, grams	Feed efficiency[c]	Protein efficiency[c]
Concentrate		1.6	+11	+0.09	+5.5
Meal		1.6	+7	+0.06	+3.6
Concentrate		3.2	+15	+0.11	+3.5
Concentrate	Soybean protein	3.2	+11	+0.04	+1.4
Meal	Soybean protein	3.2	+5	−0.01	−0.3
Concentrate	Soybean protein	4.8	+6	−0.03	−0.6

[a] 18-Day experiment.
[b] Changes in responses represent the difference in values observed in the presence and absence of 1% of cholesterol in the diets.
[c] See footnote, Table I; values determined from data of the entire experimental period.

dietary fat involved the assumption that the available energy from the alfalfa concentrate and meal amounted to 4 and 2 calories per gram, respectively. The addition of the higher level of dietary fat was made at the expense of the starch ingredient.

To the diets containing the alfalfa protein sources, which furnished 1.6 or 3.2% of dietary nitrogen, the supplements were added, these being DL-methionine at a level of 0.4 or 0.5% of the diet, L-lysine hydrochloride at a level of 0.8%, cholesterol at a level of 1.0%, and other protein at a level to furnish 1.6% of additional dietary nitrogen. The supplements were added to the diets at the expense of the starch ingredient. The diets were compounded on an air-dry basis, the moisture levels of the different ingedients being about 6% as determined by an overnight drying at 105° C.

The vitamin mix supplied the following per kilogram of diet for Experiment 1: vitamin A, 22,000 I.U.; vitamin D, 2500 I.U.; α-tocopherol, 0.13 gram; ascorbic acid, 1.13 grams; inositol, 0.13 gram; choline chloride, 1.9 grams; menadione, 0.6 gram; *p*-aminobenzoic acid, 0.13 gram; niacin, 0.1 gram; riboflavin, 0.03 gram; pyridoxine hydrochloride, 0.03 gram; thiamine hydrochloride, 0.03 gram; calcium pantothenate, 0.08 gram; biotin, 0.05 mg.; folic acid, 2.3 mg.; vitamin B_{12}, 0.13 mg.

For Experiments 2 and 3 the fat-soluble vitamins were fed separately from the diet as an oil preparation dissolved in Wesson oil. The preparation, as made from high potency fish liver oil and pure α-tocopherol acetate, supplied about 500 I.U. of vitamin A, 70 I.U. of vitamin D, and 0.65 mg. of α-tocophero acetate per two drops, which was the weekly dose per animal. Other vitamins supplied per kilogram of diet in Experiments 2 and 3 were as follows; inositol, 1.0 gram; menadione, 0.03 gram; *p*-animobenzoic acid, 0.30 gram; nicotinic acid, 0.02 gram; riboflavin, 6.0 mg.; pyridoxine hydrochloride, 6.0 mg.; thiamine hydrochloride, 0.6 mg.; calcium pantothenate, 0.04 gram; biotin, 0.1 mg.; folic acid, 2.0 mg.; vitamin B_{12}, 0.02 mg.

Results

In Experiment 1 (Table I), growth for the different groups varied considerably, being affected by both source and level of dietary nitrogen. Animals on alfalfa concentrate or gluten diets grew slowly as compared to those on soybean protein or casein diets. The responses from gluten were less than from the concentrate and that from soybean protein less than that from casein. The presence of the higher level of nitrogen in the diets—i.e., 3.2 as compared to 1.6%—improved the growth obtained with all of the nitrogen sources.

Feed and protein efficiency values were affected by the nitrogen source and level in a manner somewhat comparable to the effect on growth (Table I). This was especially true with diets containing 1.6% of dietary nitrogen, in which case the soybean protein and casein diets gave much greater feed and protein efficiencies than did the gluten and concentrate diets. When the nitrogen sources were used at the 3.2% nitrogen level, the differences observed between the diets were less but still evident. The feed efficiency values were higher with all diets at the higher nitrogen level, but the protein efficiency values were higher only with the gluten and concentrate diets. In the case of the soybean protein and casein diets, protein efficiency values tended to decrease at the higher level of dietary nitrogen. This effect has been observed previously with high quality protein sources (*14*).

Additions consisting of the amino acids, methionine and lysine, to the alfalfa concentrate diet (Experiment 2, Table II) were beneficial for growth and feed utilization. Growth was increased 25 grams by methionine alone and 45 grams by a supplement of both methionine and lysine. Feed and protein efficiencies were likewise increased by the additions of these amino acids, and the values for the concentrate diet containing both amino acids as added supplements were greater than those obtained for the diet containing soybean protein as a single nitrogen source.

The use of protein as the nitrogen supplement to the alfalfa concentrate diet also produced large improvements in growth and feed utilization. Of the soybean protein or fish meal supplements, the soybean protein produced somewhat greater improvements in growth and feed utilization. The presence of methionine as a supplement together with the soybean protein produced an effect much greater than that obtained from the soybean supplement alone, thereby suggesting that a methionine deficiency existed in the soybean supplemented diet.

Animals receiving the alfalfa meal diet in Experiment 2 grew slightly better than those on the concentrate diet, but responded to supplements of methionine and various protein supplements in much the same manner as the concentrate groups. Only a small response from lysine supplementation was observed, however, an effect which differed from that obtained with the concentrate diet.

The addition of cholesterol to the alfalfa diets (Experiment 3, Table III) improved the growth and feed utilization obtained with the diets containing the concentrate or meal in most instances. The improvements were generally small but definite when the diets were without a supplemental protein source, in which case both the growth and feed utilization were improved. When a supplement—i.e., soybean protein—was present in the diets, a small growth response was still evident but the feed efficiency and protein efficiency values were not noticeably changed.

Discussion

The experiments suggest that the protein of the alfalfa concentrate is inadequate for rats when unsupplemented and greatly improved by supplementation with certain amino acids, namely methionine and lysine. These amino acids have been reported to be deficient in alfalfa or in an alfalfa concentrate (*3*, *10*, *15*), and the present work indicated that both are deficient in some alfalfa products, with methionine being the more limiting. Another amino acid deficiency, isoleucine, has been postulated with herbage protein (*2*), but a deficiency of this amino acid was not found with the alfalfa concentrate or meal in the studies at this laboratory (*7*). However, other amino acids may be limiting in the alfalfa concentrate, since the total growth observed with both methionine and lysine supplements was less than that obtained on a casein diet.

The good responses obtained from supplementing the alfalfa concentrate or meal diets with other protein sources

were of interest. These suggested that the alfalfa nitrogen may be utilized rather well in practical diets. Because the addition of methionine to the soybean supplemented diet gave a response above that obtained from the soybean protein, the amino acid compositions of supplements used with alfalfa protein is evidently an important consideration.

The use of alfalfa meal in some of the experiments, although with diets different from those used with the concentrate, suggested that the protein quality of the meal source was quite similar to that of the concentrate. The poor response from the meal diet when not supplemented with certain amino acids or protein sources and the responses obtained in the presence of these supplements supported this contention. The response from lysine supplementation with the concentrate but not with the meal diet was of interest. This observation suggested that the meal diet was not deficient in lysine. Because lysine of protein is known to be sensitive to heat treatments under certain conditions (*4*), it is possible that the heat treatment involved with the preparation of the concentrate was responsible for this difference between the two alfalfa diets.

The lower degree of growth and feed utilization obtained with the meal than with the concentrate diet when methionine and lysine supplements were both present is not readily explained. The possibility that this effect may be related to a lower content of protein nitrogen in the meal diet than in the concentrate diet is presented. This contention is supported by data which show that as much as 20% of the nitrogen of unprocessed alfalfa may consist of nonprotein components (*6*).

The beneficial effects obtained from the addition of cholesterol to the alfalfa diets may be attributable to the presence of saponins in the alfalfa concentrate and meal. Studies have indicated that extracts of alfalfa tend to foam strongly (*11*) and to give addition-complexes in the presence of cholesterol (*16*); these properties have long been associated with the presence of saponins in aqueous solution (*5*). Further, the growth-depressant effect which occurs upon the addition of the alfalfa extracts to adequate diets has been shown to be largely counteracted by the presence of cholesterol in the diet (*11*). Since the growth-stimulatory effects observed from cholesterol in the present experiments were small, it is not likely that saponins interfered in the present protein evaluation tests to an important extent. The observation that the cholesterol effects were greatest with the unsupplemented diets is as expected, since a small increase in feed intake with such low quality diets can be reflected as a definite increase in growth and feed utilization.

With the present experiments showing that the protein concentrate from alfalfa was utilized quite well under certain conditions by rats, a need remains for making tests on the concentrate with animals such as poultry and swine. These animals are known to be adapted to low-fiber diets on which they are highly efficient for meat production. Tests with these animals should involve comparisons of the protein of the concentrate with that of alfalfa meal and alfalfa leaf meal. In delineating these future tests, it is recognized that the nutrient quality of the alfalfa concentrates may be improved and that there may be beneficial changes made in the protein and other nutrients of alfalfa meals. In making the tests it is expected that difficulties will be encountered with certain of the plant ingredients such as growth depressions from saponin (*8, 11*), egg white discoloration from chlorophyll (*9*), and undesirable responses which might be attributable to the high fiber content of alfalfa meals (*8*). However, a successful completion of animal tests on the quality of alfalfa concentrates and other alfalfa products is needed if large-scale production of alfalfa concentrates is to be approached. The use of a crop such as alfalfa with its great potential for protein production as a source of protein concentrate could be instrumental in enabling expansions of meat production in the future.

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