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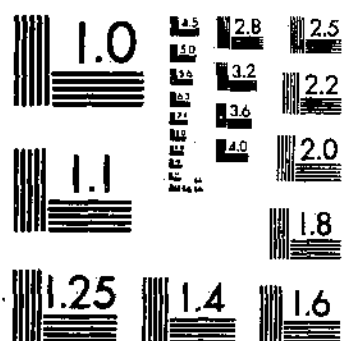
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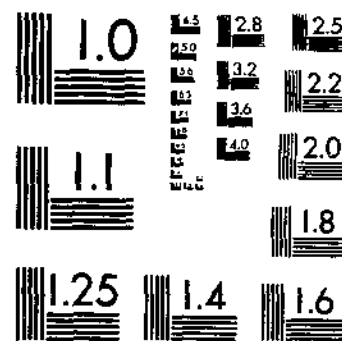
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Washington, D.C.

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Composition of Dehydrated Forages

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INTRODUCTION

A constant demand exists for information concerning forage constituents, especially with regard to changes in constituents with stages of growth and to alterations brought about by processing practices; for example, pelleting and regrinding. Many workers have reported increased weight gains when ruminants were fed pellets instead of dehydrated meals. The cause of this apparent growth stimulation is not clear, and this is one of the factors that prompted the present study.

A great quantity of compositional data of forages has been reported. With financial support from the U.S. Department of Agriculture, the National Research Council of the National Academy of Sciences compiled and published all available data (24).² Similar financial support was given by the Western Utilization Research and Development Division to the Colorado Agricultural Experiment Station for a study of the storage stability of vitamins E and K, and β -carotene in dehydrated alfalfa. This investigation also included a comparison of first- and third-cutting alfalfa with respect to the content and stability of these nutritive factors, a comparison of commercially dehydrated and of sun-cured alfalfa, and a spot check of open-market samples of dehydrated alfalfa (24). Many other studies of a similar nature have been carried out in laboratories concerned with forage composition and utilization (3, 18, 19). However, many data that are available do not permit adequate correlations, because, in most of the studies, different constituents have been determined in different samples of a forage. It was therefore decided to select a limited number of samples and study these intensively. In any such study a maze of possible analytical schemes is encountered from which it is necessary to choose one that will be most satisfactory for the purposes of the investigation.

For several years many workers who have been concerned with the task of assessing the feeding value of forages have been dissatisfied

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² Italic numbers in parentheses refer to Literature Cited, p. 19.

with the information obtained from the traditional scheme of proximate analysis (3, 11, 14, 26, 33). The shortcomings of this scheme are well known. (1) Fractions determined as crude fiber and nitrogen-free extract are not chemically uniform (26, 32). (2) These fractions contain varying percentages of their many components, depending upon the type of plant material analyzed and even upon the stage of growth when considering a single plant species (26, 33). (3) Whereas the digestibility of crude fiber should theoretically be lower than that of the nitrogen-free extract, digestion trials have shown the reverse to be true in almost 40 percent of the cases examined (3). The proximate analysis scheme is not without some value, but its many limitations must be recognized.

Many new or revised analytical schemes have been proposed (12, 13, 14), but it is not the intention in this bulletin to attempt an evaluation or critique of these proposals. Nor is it implied that all the procedures used in this study should be adopted by other workers in the field. Rather, data obtained by several analytical methods are presented, so that each worker may be better able to select that combination of techniques which will provide him with the information most suitable to his investigations.

However, some of the data obtained in this study strongly suggest that a rather simple procedure may be used for the indirect estimation of lignin and total cell-wall material, especially where these quantities are desired as indicators of the nutritive value of dehydrated forage. Also, a determination of the extent of either beneficial or harmful effects of pelleting and regrounding dehydrated alfalfa meal (common commercial practices) on the vitamin and other chemical components of the forage is attempted. For this study six samples of dehydrated alfalfa meal were pelleted, including two that were treated with an antioxidant (ethoxyquin, 1, 2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) (9) dispersed in 1 percent and 5 percent animal tallow. The analytical data obtained from these samples are included for their value as replicates.

Of the 24 samples analyzed, 12 were dehydrated alfalfa meals, 6 were reground pellets (made from alfalfa meal), and the 4 grass and 2 grass-legume mixtures were in the form of dehydrated meals. The samples were obtained from a number of geographical locations so that the analytical data might be more generally applicable. Care was taken to obtain a fairly complete agronomic history of each sample, and this history plus the extensive analyses performed on individual samples should increase the value of the data presented.

SAMPLE INFORMATION

Agronomic History

In order to obtain a wide spread in the "quality" of samples, each cooperating alfalfa dehydrator was asked to provide four samples of dehydrated alfalfa meal containing approximately 13, 17, 21, and 25 percent protein, respectively. Because such a wide range of "quality" is not ordinarily available, it was necessary to collect the samples over several seasons and to cut the alfalfa at earlier or later stages of

crop maturity than is usual, in order to obtain, respectively, the high and the low "quality" samples.

An extensive agronomic history was obtained for each sample used in this study. It is possible that information concerning the extent of fertilization, irrigation, and other cultural practices may help to explain certain differences in chemical composition of the forage samples. For this reason, each supplier of sample material was requested to complete a form listing as much information as was available concerning the sample. A compilation of this information is presented in table 1.

Processing and Storage

Harvesting, chopping, dehydrating, and grinding were all performed by commercial dehydrators who used their normal commercial procedures. The samples were bagged and shipped to the Laboratory by railway freight. Unavoidable delays en route resulted in considerable variation in the time required for shipping; in some cases this amounted to as much as 4 weeks. In addition, sample O-3 was stored, unrefrigerated, for 3 months prior to shipment. All alfalfa samples were stored (refrigerated) for periods as long as a year prior to performing some vitamin and other chemical analyses. For these reasons certain indexes used to judge the quality of forages, e.g., carotene and xanthophyll content, are not comparable and should not be considered to represent true values for freshly dehydrated alfalfa. These data have been included for the sake of completeness, but they also serve as examples of quality loss that may result from prolonged storage.

When the bagged samples arrived at the Laboratory, they were transferred to 5-gallon cans and covered with tightly fitting friction lids. The cans were immediately stored at 0° F. Most of the meals as received would pass through 40-60 mesh screens; meals of larger particle size were ground to this mesh in a Wiley mill prior to analysis.

Portions of samples N-1, N-2, N-3, and N-4 were pelleted with a laboratory-scale pellet mill, wherein a rotating impeller forced the meal through holes in a rotating steel die. The pellets produced by this die were approximately $\frac{3}{16}$ inch in diameter and about $\frac{1}{2}$ inch long. It was necessary to add 3 to 5 percent water to the meals in order to make pelleting possible, since the mill was not equipped with steam injection as are commercial-size pellet mills.

Other portions of sample N-3 were pelleted but an antioxidant and animal tallow were added. The antioxidant and tallow were applied by slurring the meal in Skellysolve B³ containing both the antioxidant and the tallow. The slurry was mixed constantly until all solvent had evaporated. The antioxidant used was ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline), and the tallow used was high-grade animal tallow.

³ Manufacturers should consult the Food and Drug Administration, Washington 25, D.C., and the food and drug officials of the individual States involved, to determine if the use of any proposed additive is permissible, and if so, what limitations are placed on its use.

TABLE 1.—Agronomic history

Sample and No.	Common plant or varietal name	Genus and species	Where grown	Age of stand	Date cut	Cutting	Time from previous cutting	Yield
				Years			Days	Tons per acre
Dehydrated alfalfa:								
N-1	Nebraska Common	<i>Medicago sativa</i> ..	Cozatl, Nebr..	4	7-30-56	2d	50	1.5
N-2	do.	<i>Medicago sativa</i> ..	do.	6	9-3-56	3d	43	1.25
N-3	Ranger	<i>Medicago sativa</i> ..	do.	3	9-24-56	4th	40	1.2
N-4	do.	<i>Medicago sativa</i> ..	do.	4	10-18-56	4th	41	.4
C-1	California Common	<i>Medicago sativa</i> ..	Dixon, Calif..	2	8-20-56	5th	62	1.75
C-2	do.	<i>Medicago sativa</i> ..	do.	2	8-27-56	5th	34	1.1
C-3	do.	<i>Medicago sativa</i> ..	do.	2	7-23-56	4th	30	.75
C-4	do.	<i>Medicago sativa</i> ..	do.	2	9-15-56	6th	36	1.3
O-1	Kansas Common	<i>Medicago sativa</i> ..	Graytown, Ohio.	1	7-1-57	1st	0	1.75
O-2	Ranger	<i>Medicago sativa</i> ..	do.	1	9-5-56	3d	33	1.25
O-3	do.	<i>Medicago sativa</i> ..	do.	2	6-21-60	2d	38	1.0
O-4	do.	<i>Medicago sativa</i> ..	do.	1	5-8-57	1st	0	.6
Dehydrated alfalfa, pelleted and reground:								
N-1-P ¹	Nebraska Common	<i>Medicago sativa</i> ..	Cozatl, Nebr..	4	7-30-56	2d	50	1.5
N-2-P ¹	do.	<i>Medicago sativa</i> ..	do.	6	9-3-56	3d	43	1.25
N-3-P ¹	Ranger	<i>Medicago sativa</i> ..	do.	3	9-24-56	4th	40	1.2
N-4-P ¹	do.	<i>Medicago sativa</i> ..	do.	4	10-18-56	4th	41	.4
N-3-P-E-1 ¹	do.	<i>Medicago sativa</i> ..	do.	3	9-24-56	4th	40	1.2
N-3-P-E-5 ¹	do.	<i>Medicago sativa</i> ..	do.	3	9-24-56	4th	40	1.2
Dehydrated grass and grass-legume meals:								
W-1	Reed canary-grass.	<i>Phalaris arundinacea</i> .	Snobomish, Wash.	6	6-8-57	2d	20	.9
W-2	Common rye-grass.	<i>Lolium</i> sp.	do.	1	6-15-57	1st	0	
A-1	Balbon rye.	<i>Secale cereale</i> ..	Fort Smith, Ark.	60	11-1-57	1st	0	.6
Oa-1	Orchardgrass.	<i>Dactylis glomerata</i> .	Wallaceburg, Ontario, Canada.	6	6-21-58	3d	17	.85
Oa-2 ¹	Orchardgrass and Ladino whiteclover.	<i>Dactylis glomerata</i> , <i>Trifolium repens</i> .	Chilliwack, British Columbia, Canada.	2	9-2-58	8th	15	
W-3 ¹	Orchardgrass, Ladino whiteclover, Alta fescue.	<i>Dactylis glomerata</i> , <i>Trifolium repens</i> , <i>Festuca arundinacea</i> .	Burlington, Wash.	2	9-13-58	8th	29	1.15

¹ Lined in 1951.² Same as dehydrated sample of same number.³ Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) and 1 percent tallow added and reground.⁴ Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) and 5 percent tallow added and reground.⁵ In days.⁶ Orchardgrass, 81.8 percent; Ladino whiteclover, 18.2 percent.⁷ Orchardgrass, 74.5, Ladino whiteclover, 12.5, and Alta fescue, 13.0 percent.

of dehydrated forage samples

Condition of crop when cut	Previous crop	Fertilizer on—		Soil acidity	Soil type	Irrigation
		Current crop	Previous crop			
Full bloom, leafy, stemmy.	Corn	0	0	pH	Hall silt loam	No.
do	do	0	do		do	Yes.
Bud, leafy	do	0	do		Fine sandy loam	Yes.
Bud, very leafy	do	0	do		Hall silt loam	No.
Full bloom, stemmy.	Sugar beets	0	NH ₃		Capay silty clay loam.	Yes.
Bud, very leafy	Barley	0	NH ₃ (1953)		do	Yes.
do	do	0	NH ₃		do	Yes.
do	Sugar beets	0	do		Yolo silty clay loam.	Yes.
Stemmy	Wheat	0	0		Toledo clay loam.	No.
Bud, leafy	do	0	300 of 5-10-10	6.8	Brookstone clay	No.
1/4 bloom, leafy	do	150 of 3-12-12	300 of 3-12-12	6.8	do	No.
Prebud	do	200 of 5-20-20	300 of 5-20-20	6.6	Toledo clay loam	No.
Full bloom, leafy, stemmy.	Corn	0	Nitrogen		Hall silt loam	No.
do	do	0	do		do	Yes.
Bud, leafy	do	0	do		Fine sandy loam	Yes.
Bud, very leafy	do	0	do		Hall silt loam	No.
Bud, leafy	do	0	do		Fine sandy loam	Yes.
do	do	0	do		do	Yes.
Leafy	Grass	200 of 11-48-0+200 of muriate potash.	As on current crop + N and K after cuts.		Peat muck	No.
Leafy, some stems	do	do	do		do	No.
About 8 weeks old.	Alfalfa	200 of ammonium nitrate.			Sandy loam	Yes.
Very leafy	do	200 of ammonium nitrate after each cut.	1,000 of 12-12-12 (5 treatments).	7.2	Thamesville clay loam.	Yes.
Leafy, 10-12 inches.	do	48.6 N+25.2 P	300 of N+150 of P+90 of K.		Sandy loam	Yes.
Leafy, 14-10 inches	Peas	100 of 46 percent superphosphate +70 of muriate potash +70 of sulfate potash +500 of ammonium nitrate in 4 applications.		5.7	Clay loam (river bottom).	Yes.

All pelleted samples were reground in the laboratory to 40-60 mesh. The reground pellets were also stored in 5-gallon cans at 0° F.

METHODS OF ANALYSIS

Proximate Analyses

1. *Moisture*.—Exactly weighed samples, 1 to 2 g., in aluminum moisture dishes, heated 4 hours at 100° to 105° C., cooled in a desiccator and weighed.

2. *Grit*.—By official A.O.A.C. (Association of Official Agricultural Chemists) method (4), except that carbon tetrachloride was used instead of chloroform (18).

3. *Crude fat*.—Ethyl ether extractives, determined by the official A.O.A.C. method (4).

4. *Ash*.—By official A.O.A.C. method (4).

5. *Protein*.—Kjeldahl-Walker-Gunning method, A.O.A.C. (4).

6. *Crude fiber*.—By official A.O.A.C. method (4).

Summative and Organic Acid Analyses

1. *Nonvolatile organic acids*.—The method of Palmer (28) was used. The acids present in a hot water extract of the sample were eluted from a formate resin with 6-normal formic acid. Collected fractions of the eluate were evaporated, taken up in water, and titrated with 0.0125-normal sodium hydroxide. The rates of elution and evaporation had to be carefully controlled and kept nearly constant from one sample to another, in order to obtain reproducible titration data. In this study no attempt was made to separate and identify the individual acids, so that the data report only "total non-volatile organic acids" as obtained by this method. Nor does the method include oxalic acid, because this acid would not be obtained in a water extract of the plant material.

2. *Ethanol-benzene azeotrope solubles*.—For this determination, a portion of each sample, in a paper thimble, was extracted in a Soxhlet apparatus with the azeotrope of ethanol-benzene (1:2, by volume) as the solvent. A large Soxhlet extractor was used, and Raschig-type glass rings at the bottom of the extractor insured complete drainage of the solvent from the thimble. Reflux was maintained for 72 hours. After evaporation of solvent from the residue, the weight lost by the sample was determined, and this was taken as ethanol-benzene solubles. These data checked closely with those obtained by evaporating the solvent and weighing the extracted matter.

3. *Ammonium oxalate solubles*.—This assay was performed on the residue obtained after extraction of the sample with the ethanol-benzene azeotrope. The method used was a modification of that of Henderson (15) and involved a 4-hour and a 16-hour extraction of the plant material at 85° C. with 10 volumes of 0.5 percent aqueous ammonium oxalate. The residue was filtered and washed with hot water after each extraction, and finally air-dried; the moisture content and weight loss were then determined. This weight loss cannot be considered as "crude pectin," because considerable water-soluble material

is included and, therefore, only the designation "ammonium oxalate solubles" has been used.

4. *Sodium chlorite holocellulose*.—The material on which this assay was performed was that final residue obtained after successive extraction with the ethanol-benzene azeotrope and ammonium oxalate, as described above. The method used was that of Whistler and coworkers (38). Samples were treated at 85° C. for 1 hour, with 4 additions of acetic acid and sodium chlorite. Extensive cold water washing of the holocellulose residue on the filter was followed by acetone and ethyl ether rinses. The holocellulose was then air-dried, and moisture and yield were determined.

5. *Pentosan*.—Pentosan material was determined in the chlorite holocelluloses prepared as above. The method of Adams and Castagne (1) was used to determine the quantity of furfural derived from pentose upon reflux of the sample in 12 percent hydrochloric acid. An Evelyn photoelectric colorimeter, fitted with a 515-millimicron filter, was used for measurement of the color produced by the reaction of furfural with an aniline acetate reagent. The furfural measured was multiplied by the factor 1.55, to convert it to pentosan, with the assumption that all of the pentose was xylose and without correction for furfural derived from uronic acids. The distillation of furfural from pentose material is empirical and must be carefully controlled. However, the formation and measurement of the furfural-aniline acetate color complex are quite accurate and reproducible, and the method is much less tedious than the older phloroglucin procedure (4).

6. *Lignin*.—The method of Norman and Jenkins (27), modified so as to substitute autoclaving for refluxing (Binger and Norman (10)), was used for the determination of lignin, both in the original samples and in the chlorite holocellulose preparations. A finely woven nylon cloth was used for filtration, because nylon was found to be less affected by repeated exposure to acid and it retained fine particles better than the lawn (cotton broadcloth) specified in the procedures cited above.

Vitamin Assays

1. *Pantothenic acid*.—A.O.A.C. method (4).
2. *Choline*.—Method of Horowitz and Beadle (17).
3. *Pyridoxine*.—Method of Atkin and others (6).
4. *Inositol*.—Method of Atkin and others (6).
5. *Riboflavin*.—A.O.A.C. method (4).
6. *Niacin*.—A.O.A.C. method (4).
7. *Folic acid*.—A.O.A.C. method (4).
8. *Thiamine*.—Method of the Association of Vitamin Chemists, Inc. (5).
9. *Tocopherols* (total).—Colorimetric—(a) saponification in the presence of an antioxidant; (b) removal of vitamin A and β -carotene (Parker and MacFarlane) (29); (c) determination of tocopherol (Quaife and Harris) (30).
10. β -*carotene*.—A.O.A.C. method (4).
11. *Xanthophylls*.—Method of Bickoff and coworkers (8).
12. *Vitamin K*.—Method of Almquist (2).

13. *Betaine*.—A modification of the method of Beattie (7) was further modified by the workers of the Wisconsin Alumni Research Foundation, as detailed after the following outline of the modified original method: The sample was refluxed with barium hydroxide in 20 percent aqueous methanol to remove ammonia, and then extracted and made to volume in methanol. Choline was separated by passing a part of the extract through potassium decalzo. The eluate was then passed through barium decalzo, to retain the betaine. After elution with water, the betaine was precipitated with Reinecke salt in dilute phosphoric acid solution. The precipitate was separated, washed, dissolved in acetone, and read in a spectrophotometer.

The procedure was used as stated above but with two exceptions. (a) A standard curve was prepared on an Evelyn spectrophotometer, with a 515-millimicron filter, and all analyses were based on this standard curve. (b) It was advisable to decolorize the extracts with norit, to adapt this procedure to forage extracts, which are highly colored. Decolorization removed a substantial quantity of interfering material, which otherwise caused difficulty on the decalzo columns.

Calculation of Data

Moisture and grit were determined in air-dry material as received, and are reported as "percentage of air-dry weight." All subsequent analytical data were then calculated on the basis of "moisture- and grit-free original material."

The factor 6.25 was used to convert Kjeldahl nitrogen to protein, and the other proximate analyses were calculated as described in the Official Methods of Analysis of the A.O.A.C. (4).

Nonvolatile organic acids were calculated as "millicequivalents of acid per gram of moisture- and grit-free original material." Because neither the identity nor the percentages of these individual acids were determined, only an approximation of the percentage content of non-volatile acids is possible. For the alfalfa samples, an average equivalent weight of 80 was used. This figure was calculated from data reported by Richardson and Hulme (37) and from data obtained by an analysis of one of the samples of this series, which was performed by D. F. Houston of the Western Utilization Research and Development Division. The average equivalent weights obtained from the data of Hulme and Houston were, respectively, 71 and 92. These data illustrate the variation possible in compositional data even when dealing with a single species.

For an approximation of the percentage content of nonvolatile organic acids in the grass samples, an average equivalent weight of 105 was used. This figure was derived from data for perennial ryegrass (*Lolium perenne*) reported by Hirst and Ramstad (16). For grass-legume mixtures, appropriate percentages of 105 and 80 were used to estimate average equivalent weights. It must be emphasized that percentage contents of acid calculated by these average equivalent weights are merely rough estimations and should not be used as absolute data.

In calculating ethanol-benzene solubles, the data for the dry-weight losses incurred by the samples upon extraction with the azeotrope were converted to percentages of moisture- and grit-free original material.

The data for the weight lost by the azetropo-extracted residues upon subsequent extraction with ammonium oxalate were converted in the same manner to ammonium oxalate solubles.

Chlorite holocellulose was calculated from the weight of material remaining after dissolution of lignin with sodium chlorite.

The content of lignin in the samples analyzed was calculated in the following manner: After the several acid hydrolyses had removed carbohydrate material, a residue was obtained that contained the lignin and mineral fractions. An exact weight was obtained, the residue was ignited at 600° C. overnight, and the loss in weight resulting from this ignition was considered to be lignin.

All vitamin data were converted to, and are presented as "milligrams per 100 g. of moisture- and grit-free original material."

RESULTS

Proximate Constituents

The data obtained by a proximate analysis of the 24 samples in the study are shown in table 2. The data for the moisture and grit analyses have been included so as to enable the reader to convert any data to other bases if so desired. In general, the relationships shown in these data, e.g., the inverse relationship of protein to crude fiber, are similar to those found by proximate analysis of most forage samples.

TABLE 2.—*Proximate analysis of alfalfa meals, reground pelleted alfalfa, and grass and grass-legume mixture meals*

Sample	Moisture ¹	Grit ¹	Protein ²	Crude fat ²	Crude fiber ²	Ash ²	Sugar (as glucose)	
							Reducing ²	Total ²
Alfalfa meal:	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
N-1	5.32	0.87	16.1	2.23	36.6	10.2	0.72	1.06
N-2	4.88	.82	15.9	2.62	31.3	11.2	1.20	3.31
N-3	6.28	1.50	21.7	4.05	24.6	12.7	.87	3.33
N-4	5.20	1.77	25.0	4.23	17.2	14.2	.91	3.12
O-1	9.10	.45	14.7	3.97	35.5	7.7	1.44	4.54
O-2	8.22	.20	18.7	2.02	20.1	8.0	1.30	3.45
O-3	7.16	.20	23.6	4.13	22.1	9.7	.78	1.65
O-4	9.43	.12	21.4	3.76	26.7	8.9	1.50	3.32
O-5	6.70	.40	15.1	2.46	35.0	7.3	1.36	2.60
O-6	8.28	.15	20.8	3.61	24.7	8.4	1.31	2.87
O-7	8.23	.15	20.3	3.44	27.8	8.3	1.42	3.16
O-8	7.15	.27	25.9	3.37	17.2	9.5	2.76	5.51
Reground pelleted alfalfa:								
N-1-P	8.27	.20	16.1	2.88	36.7	10.0	.71	1.82
N-2-P	9.02	.30	16.2	3.21	30.4	9.1	1.03	3.01
N-3-P	9.16	.40	22.1	4.63	22.2	12.1	.77	3.00
N-4-P	8.15	.80	24.3	5.43	17.1	13.5	.79	3.73
N-4-P-E-1	8.75	.03	22.2	5.07	22.6	13.5	.74	2.94
N-4-P-E-5	9.15	.02	21.3	9.74	22.9	12.8	.70	2.80
Grass and grass-legume mixture meals:								
Reed canarygrass	7.09	.20	21.8	5.11	20.7	8.2	2.39	5.70
Common ryegrass	8.75	.83	27.4	5.93	18.4	11.5	3.55	7.08
Rye	7.20	2.35	31.3	7.24	16.0	12.6	2.24	6.34
Orchardgrass	9.02	.13	25.0	5.70	22.6	10.3	1.86	4.30
Orchardgrass-Ladino								
whiteclover	7.50	1.20	27.4	5.99	19.0	10.5	2.73	7.95
Orchardgrass-Ladino								
whiteclover-fescue								
grass	8.23	.31	24.1	6.67	21.4	8.0	3.64	8.17

¹ Percentage of air-dry material.

² Percentage of moisture-free and grit-free original material.

When alfalfa meal samples N-1 through N-4 are compared with their pelleted counterparts N-1-P through N-4-P, it appears that most of the proximate constituents have been unaffected by the pelleting and regrinding processes. The exception to this is the greater quantity of crude fat found in the reground pellets as opposed to the meals. The average level of fat in the pellets was 22.3 percent greater than in the meals. This finding has previously been reported by Lindahl and Davis (20) and more recently confirmed by Lindahl and Reynolds (21). These authors postulated that at least part of the observed increase in crude fat may result from cellular rupture during pelleting, which permits greater penetration by the solvent. Lindahl and coworkers found, however, that this increase in apparent crude fat did not affect the gross energy content of the pelleted alfalfa as compared to the same alfalfa in the form of meal.

Summative Constituents and Organic Acids

Tables 3 and 4 show the quantities of the various constituents that were assayed in the so-called summative analysis scheme. The calculation of percentage content of the nonvolatile organic acids (table 3) has already been described. In the extraction of the samples with ethanol-benzene azeotrope and ammonium oxalate, large amounts of material were removed by the double extraction procedure. Thus, it was found that from 20 to 40 percent of the crude protein present in

TABLE 3.—*Summative analysis (soluble constituents) and organic acids of alfalfa meals, reground pelleted alfalfa, and grass and grass-legume mixture meals*¹

Sample	Nonvolatile organic acids		Azeotrope solubles	Oxalate solubles	Residue from 2 extractions	Crude protein in residue	Protein-free doubly extracted residue
	Meg.	Percent ²	Percent	Percent	Percent	Percent	Percent
Alfalfa meal:							
N-1	0.402	3.22	16.8	18.6	64.7	9.6	54.8
N-2	.359	2.87	18.6	19.0	61.8	12.3	46.6
N-3	.664	5.31	19.9	22.6	57.3	16.9	40.4
N-4	.684	5.47	20.8	25.8	53.4	19.6	33.8
C-1	.400	3.20	21.3	16.7	62.0	10.0	52.0
C-2	.476	3.81	19.6	19.5	60.9	12.8	48.1
C-3	.494	3.95	22.3	30.6	47.2	17.3	29.0
C-4	.540	4.32	21.8	20.0	49.2	14.2	35.0
O-1	.311	2.46	17.8	21.8	60.4	9.7	50.7
O-2	.406	3.06	20.2	27.4	52.4	14.6	37.8
O-3	.462	3.70	20.2	25.0	53.9	12.8	41.1
O-4	.636	5.09	24.9	30.1	45.0	17.7	27.3
Reground pelleted alfalfa:							
N-1-P	.400	3.20	15.0	18.6	65.5	10.7	54.8
N-2-P	.355	2.84	18.0	21.1	60.9	11.1	49.8
N-3-P	.650	5.25	21.0	22.0	50.4	13.4	43.0
N-4-P	.677	5.42	21.9	26.5	51.6	13.9	37.7
N-3-P-E-1	.643	5.14	20.8	23.1	56.1	16.9	30.2
N-3-P-E-5	.616	4.93	23.9	21.4	51.7	15.3	36.4
Grasses and grass-legume mixture meals:							
Reed canarygrass	.212	2.23	25.0	23.9	51.1	10.1	35.0
Common ryegrass	.291	3.06	25.5	25.3	49.2	17.4	31.8
Rye	.532	5.59	28.6	20.0	50.8	20.7	30.1
Orchardgrass	.299	3.14	23.8	18.7	57.5	17.9	39.6
Orchardgrass-Ladino whiteclover	.255	2.55	20.5	20.8	52.7	19.1	33.6
Orchardgrass-Ladino whiteclover-lesestegrass	.359	3.05	25.9	19.7	54.4	16.9	37.5

¹ All data based on moisture- and grit-free original material.

² Assuming an average equivalent weight of 80 for samples N-1 through N-3-P-E-5, 105 for samples of grass meals, 100 for sample of orchardgrass-Ladino whiteclover mixture, and 102 for the last sample (see text).

the original sample material was extracted by this procedure, with an average of 32 percent. Prior to Kjeldahl analysis, the residues were tested for the presence of ammoniacal nitrogen and were found to be free of this potential source of error. Evidently, the extensive hot water washing of the residue removed any ammonia that would remain from the final ammonium oxalate treatment, an observation which confirms a recent report by Waite and Gorrod (35).

TABLE 4.—*Summative analysis (insoluble constituents) of alfalfa meals, reground pelleted alfalfa, and grass and grass-legume mixture meals¹*

Sample	Chlorite holocellulose	Crude protein in holocellulose	Lignin in holocellulose	Corrected ² chlorite holocellulose	Pentosan in holocellulose (as xylan)	Lignin	Summation ³
	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Alfalfa Meals:							
N-1	50.0	0.1	4.0	45.9	7.2	12.0	103.1
N-2	50.3	0.3	8.0	41.0	7.1	10.5	102.0
N-3	47.4	0.7	3.1	34.3	5.5	8.0	101.0
N-4	43.8	12.4	2.8	28.0	3.8	6.2	101.0
C-1	54.0	0.7	3.0	46.2	10.3	7.9	101.1
C-2	52.4	8.4	3.0	41.0	8.0	7.4	100.3
C-3	30.3	11.0	3.0	25.3	4.6	6.1	101.2
C-4	40.2	8.8	2.5	28.8	4.8	7.0	100.8
O-1	50.2	5.7	4.0	41.5	7.8	11.7	101.5
O-2	42.9	8.8	3.0	31.1	4.6	7.9	101.2
O-3	45.3	8.1	3.2	34.0	5.6	8.2	101.1
O-4	34.8	11.3	3.1	20.4	2.8	6.3	99.4
Reground pelleted alfalfa:							
N-1-P	55.6	0.4	4.3	46.9	7.9	12.1	104.2
N-2-P	50.0	5.6	3.4	41.8	7.6	10.6	102.6
N-3-P	46.1	0.9	3.0	34.7	4.7	7.8	99.5
N-4-P	40.8	11.5	2.6	26.7	3.3	7.0	98.0
N-3-P-E-1	45.4	10.0	3.2	32.2	4.8	7.9	100.9
N-3-P-E-5	43.0	9.3	2.8	31.5	4.7	7.6	102.7
Grasses and grass-legume mixture meals:							
Red canarygrass	43.4	10.3	1.0	31.2	7.5	5.6	101.8
Common vetchgrass	38.0	10.0	1.8	26.5	5.7	6.5	101.2
Rye	42.0	13.4	1.8	26.8	6.7	3.4	100.1
Orchardgrass	47.6	10.9	1.3	35.4	5.6	4.8	100.6
Orchardgrass-Ladino whiteclover	42.5	11.0	1.2	30.3	4.3	4.0	100.7
Orchardgrass-Ladino whiteclover-fescuegrass	43.8	9.6	1.2	33.0	5.3	4.3	99.8

¹ All data as percent of moisture- and grit-free original material.

² Corrected for content of crude protein and lignin.

³ Summation = protein + total extractives (corrected for crude protein) + lignin + corrected holocellulose (= cellulose + pentosan).

The data in table 4 illustrate one of the problems encountered in attempts to use the sodium chlorite holocellulose assay to determine cell-wall material (12). Residual crude protein and lignin may constitute as much as 40 percent of the chlorite holocellulose isolated from the forage samples. The quantities of both crude protein and lignin in the holocelluloses bear some relation to the amounts of those constituents in the original samples, but these are too variable to be used for prediction purposes.

The possibility of prediction of some components, however, arises from an inspection of the data in table 5. The data for total cell-wall material agree very closely with the data for protein-free, doubly extracted residue, taken from table 3. In addition, for most of the alfalfa samples, lignin as a percentage of this residue approaches a constant of 20 percent.

These observations prompted a statistical analysis of the data for all 18 alfalfa samples, the results of which are presented graphically in figure 1. Regression equations were determined, and the intercept values were found, by testing, not to be significantly different from zero and, therefore, both lines were recalculated to pass through the origin. The regression equations for these lines are shown in the figure. Also shown are the 95 percent confidence limits, within which it is seen that a 1:1 relationship may be assumed for total cell-wall material and protein-free, doubly extracted residue. The same relationship was shown for the grasses and grass-legume mixtures. In this case the regression equation was $Y=1.0199X$, which is very similar to that found with the alfalfa samples. Because only six samples were available for this study, however, the 95 percent limits were much wider, but the data in table 5 clearly show that the same 1:1 relationship exists.

The second plot in figure 1 indicates that, for dehydrated alfalfa samples, lignin may be closely estimated as 20 percent of the figure obtained for protein-free, doubly extracted residue. Inspection of

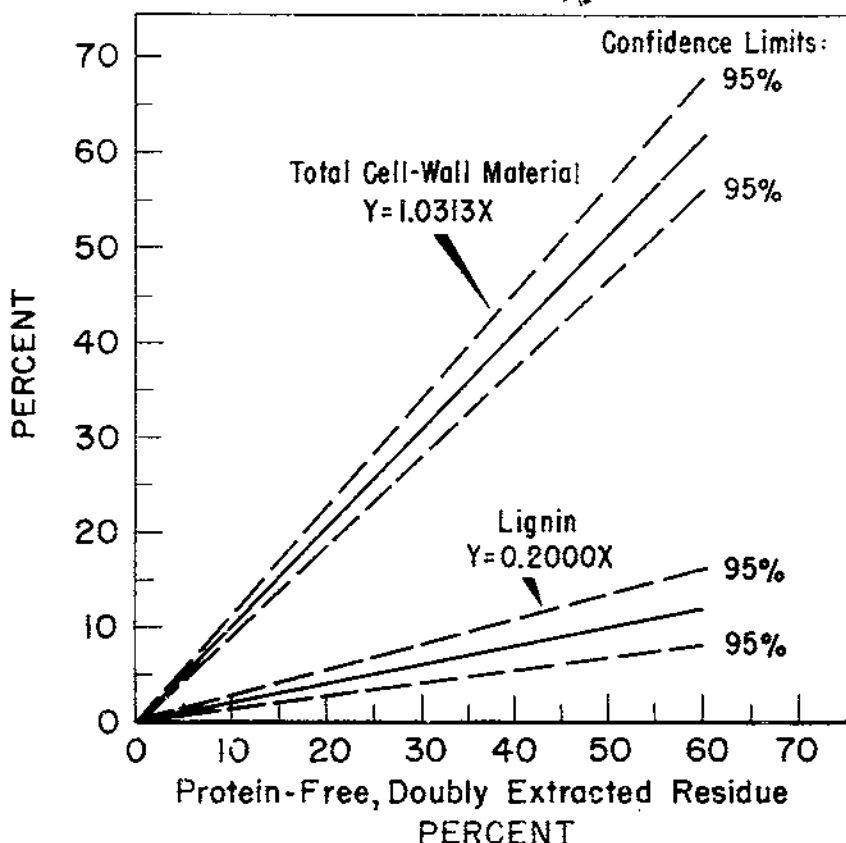


FIGURE 1.—Regression of total cell-wall material and of lignin on protein-free, doubly extracted residue of alfalfa meals and reground pelleted alfalfa, as percentage of moisture- and grit-free original material.

the data in table 5 reveals that there is a species difference in this case; for grasses, a 20-percent estimate cannot be used. It appears that a more intensive study of each species would be necessary to determine whether or not lignin is a constant percentage of the residue as it was in the alfalfa samples studied.

TABLE 5.—Comparison of certain cell-wall data with those for protein-free, doubly extracted residue¹

Sample	Cellulose ²	Pentosan	Lignin	Total cell-wall material ³	Protein-free doubly extracted residue	Lignin as percentage of protein-free doubly extracted residue
	Percent	Percent	Percent	Percent	Percent	Percent
Alfalfa meals:						
N-1	38.7	7.2	12.0	57.9	54.8	21.9
N-2	33.9	7.1	10.5	51.5	49.5	21.2
N-3	28.8	5.5	8.0	42.3	40.4	19.8
N-4	24.8	3.8	6.2	34.8	33.8	18.3
C-1	31.0	10.3	7.9	53.1	52.0	15.2
C-2	33.0	8.0	7.4	48.4	48.1	15.4
C-3	26.7	4.6	6.1	31.4	29.0	20.4
C-4	24.0	4.8	7.0	35.8	35.0	20.0
O-1	33.7	7.8	11.7	53.2	50.7	23.1
O-2	26.5	4.0	7.9	39.0	37.8	20.9
O-3	28.4	5.6	8.2	42.2	41.1	20.0
O-4	17.6	2.8	6.3	26.7	27.3	23.1
Reground alfalfa pelleted:						
N-1-P	30.0	7.9	12.1	59.0	54.8	22.1
N-2-P	31.3	7.5	10.6	52.1	49.8	21.3
N-3-P	30.0	4.7	7.8	42.5	43.0	18.1
N-4-P	25.4	3.3	7.0	35.7	37.7	18.6
N-3-P-E-1	27.4	4.8	7.9	40.1	39.2	20.1
N-3-P-E-2	26.8	4.7	7.0	39.1	36.4	20.9
Grasses and grass-legume mixture meals:						
Reed canarygrass	23.7	7.5	5.6	36.8	35.0	10.0
Common ryegrass	26.8	5.7	6.5	39.0	31.8	20.4
Rye	20.1	6.7	3.4	30.2	30.1	11.3
Orchardgrass	29.8	6.6	4.8	40.2	39.6	12.1
Orchardgrass-Ladino whiteclover	26.0	4.3	4.0	34.3	33.6	11.9
Orchardgrass-Ladino whiteclover-fescuegrass	27.7	5.3	4.3	37.3	37.5	11.5

¹ All data as percentage of moisture- and grit-free original material.

² Cellulose = corrected holocellulose minus pentosan.

³ Total cell-wall material = cellulose + pentosan + lignin (does not include pectin).

It should also be remembered that the relations shown here were obtained by using certain methods of analysis, and that the use of other analytical methods will require confirmation of these results. The double-extraction procedure, however, appears to be very promising for rapid estimation of total cell-wall material and for lignin, where extreme accuracy may not be required; for example, in determining the nutritive value of forages (Meyer and Lofgreen (23)). Another factor that must be considered is the magnitude of the grit contamination of the samples. If the grit content is 5 percent or more, it will have to be taken into account in certain of the analyses. Data for both protein-free, doubly extracted residue and total cell-wall material include most of the grit fraction. On the other hand, neither crude fiber nor lignin data include much of the grit, because their analytical procedures include ignition and compensation for the unignited residual mineral material. The samples used in this study contained relatively small quantities of grit, and so no corrections were made in the data for the protein-free, doubly extracted residue or total cell-wall material.

Vitamin Constituents

Results of the assays for 13 vitamins and accessory factors are shown in tables 6 and 7. As was explained earlier, the data for β -carotene and xanthophyll content of the alfalfa samples are not reliable, because of prolonged storage prior to assay. These data are, however, reliable for the grass and grass-legume samples, as these were analyzed within a short time of their receipt. These grass samples are obviously very rich sources of the fat-soluble vitamin factors assayed. In general, samples with higher protein content also showed a higher vitamin content. This is true not only for the fat-soluble, but also for the water-soluble vitamins and accessory factors.

TABLE 6.—*Fat-soluble vitamin factors of alfalfa meals, reground pelleted alfalfa, and grass and grass-legume mixture meals¹*

Sample	β -carotene ²	Xanthophylls ²	Total tocopherols	Vitamin K
	Mg./100 g.	Mg./100 g.	Mg./100 g.	Mg./100 g.
Alfalfa meals:				
N-1	7.4	16.3	11.7	1.23
N-2	10.3	18.2	17.3	1.11
N-3	20.3	43.7	16.5	2.09
N-4	28.7	50.3	23.4	1.78
O-1	12.7	24.9	22.8	1.97
O-2	22.0	44.0	18.1	1.82
O-3	19.8	40.2	18.9	2.04
O-4	18.5	51.5	16.9	3.11
O-1	7.9	17.6	11.4	2.12
O-2	20.0	51.5	21.7	1.87
O-3	20.0	47.0	22.2	1.93
O-4	22.4	45.8	18.1	1.70
Reground pelleted alfalfa:				
N-1-P	7.9	18.4	9.6	2.81
N-2-P	11.6	21.8	17.8	1.31
N-3-P	21.7	47.3	17.3	1.54
N-4-P	27.5	69.5	28.1	2.02
N-3-P-E-1	24.0	40.3	17.9	1.36
N-3-P-E-5	21.9	46.3	15.0	1.31
Grasses and grass-legume mixture meals:				
Reed canarygrass	36.4	69.8	24.2	1.64
Common ryegrass	45.1	105.3	23.6	1.62
Itye	39.0	144.2	34.5	6.30
Orchardgrass	45.5	108.7	31.4	3.25
Orchardgrass-Ladino whiteclover	53.1	122.5	29.3	1.93
Orchardgrass-Ladino whiteclover-fescue-grass	58.5	123.6	25.3	4.60

¹ All data as milligrams per 100 g. moisture- and grit-free original material.

² Values are low for alfalfa samples because of delay in assay (see text).

A comparison of the vitamin contents of alfalfa meal samples N-1 to N-4 with their pelleted and reground counterparts (samples N-1-P to N-4-P) showed that in only two cases did significant decreases result from the pelleting-regrinding process. Figure 2 shows this effect on the two vitamins, inositol and pyridoxine. The loss of inositol averaged about 25 percent, and the loss of pyridoxine about 30 percent—both losses were considerable and statistically significant (5-percent level). The positive correlation of the vitamins with protein, previously referred to, is also evident in the figure. Other apparent increases or decreases in vitamin content resulting from pelleting proved to be not statistically significant.

In this comparison of four dehydrated alfalfa meals with their equivalent reground pellets, only changes in inositol, pyridoxine, and

crude fat contents were detected. It does not seem likely that these changes are sufficiently important to account for the increased growth response that many investigators have reported for pellet-fed animals (22, 26, 27). This phenomenon of growth stimulation may be related to the increased bulk density of the pelleted material.

TABLE 7.—*Water-soluble vitamin factors of alfalfa meals, reground pelleted alfalfa, and grasses and grass-legume mixture meals*¹

Sample	Thi- amin	Ribo- flavin	Panto- thenic acid	Niacin	Pyri- doxine	Inos- itol	Folic acid	Cho- line	Be- taine
	Mg./ 100 g.	Mg./ 100 g.	Mg./ 100 g.	Mg./ 100 g.	Mg./ 100 g.	Mg./ 100 g.	Mg./ 100 g.	Mg./ 100 g.	Mg./ 100 g.
Alfalfa meals:									
N-1	0.28	0.802	2.507	4.30	0.657	136	0.192	50	192
N-2	.19	.820	2.842	4.00	.494	187	.191	103	356
N-3	.41	1.475	4.656	4.47	.910	298	.330	130	392
N-4	.56	1.622	6.081	5.05	.924	410	.366	137	282
C-1	.41	.832	4.097	2.92	.520	145	.148	98	221
C-2	.50	1.551	6.353	4.10	1.096	183	.417	100	263
C-3	.67	1.040	6.760	4.45	1.144	204	.380	112	263
C-4	.65	1.833	8.171	5.00	.880	195	.445	136	206
O-1	.34	.840	2.101	4.41	.606	165	.170	103	159
O-2	.54	1.621	5.217	5.68	.810	212	.301	144	225
O-3	.53	1.430	6.350	5.85	.796	194	.282	157	220
O-4	.58	1.821	3.734	6.85	.760	325	.396	238	359
Reground pelleted alfalfa:									
N-1-P	.26	.843	2.630	5.26	.406	138	.216	191	214
N-2-P	.30	.865	2.787	4.50	.377	149	.196	124	331
N-3-P	.31	1.353	4.418	4.99	.647	190	.328	126	230
N-4-P	.64	1.682	4.072	5.44	.680	225	.408	134	254
N-3-P-E-1	.42	1.355	4.680	4.34	.767	256	.340	143	204
N-3-P-E-3	.43	1.343	4.435	4.69	.668	290	.297	123	219
Grasses and grass-legume mixture meals:									
Reed canarygrass	.64	1.715	1.826	7.30	1.400	119	.286	180	149
Common ryegrass	.61	1.448	1.407	6.63	1.034	118	.272	180	80
Rye	.94	2.083	1.740	7.40	1.080	161	.500	172	1,023
Orchardgrass	.47	2.113	1.214	4.65	.955	146	.033	160	423
Orchardgrass-Ladino whiteclover	.82	1.934	1.284	6.30	1.101	233	.313	149	304
Orchardgrass-Ladino whiteclover-lescue- grass	.72	2.057	1.265	5.10	1.078	242	.252	136	284

¹ All data as milligrams per 100 g. moisture- and grit-free original material.

The analytical data obtained for the dehydrated alfalfa meal samples show definite trends that may be related to protein content. Therefore, it is possible to simplify the tabulation of data by the calculation of certain averages, which may facilitate more rapid location of the data sought. These averages (shown in table 8) were determined by combining the data of three groups of samples with similar protein contents that provide average protein values of about 15, 20, and 25 percent. Both positive and negative correlations of all constituents with protein content are readily apparent when the data are presented in this manner. Such a combination of data would, of course, be invalid with the grasses and grass-legume mixtures, as each of these samples is of a different species.

The significance of the vitamin data obtained from the 12 dehydrated alfalfa meals is best shown by such information as contained in table 9. This table indicates the extent to which alfalfa can contribute toward supplying some of the nutrients required in poultry diets. The percentages of requirements supplied by alfalfa were based on the average vitamin data for the 20.6-percent protein level (as shown in last part of table 8), and it is assumed that alfalfa meal is only 5

percent of the total ration. Alfalfa can contribute substantially toward providing many of the factors required for proper poultry nutrition.

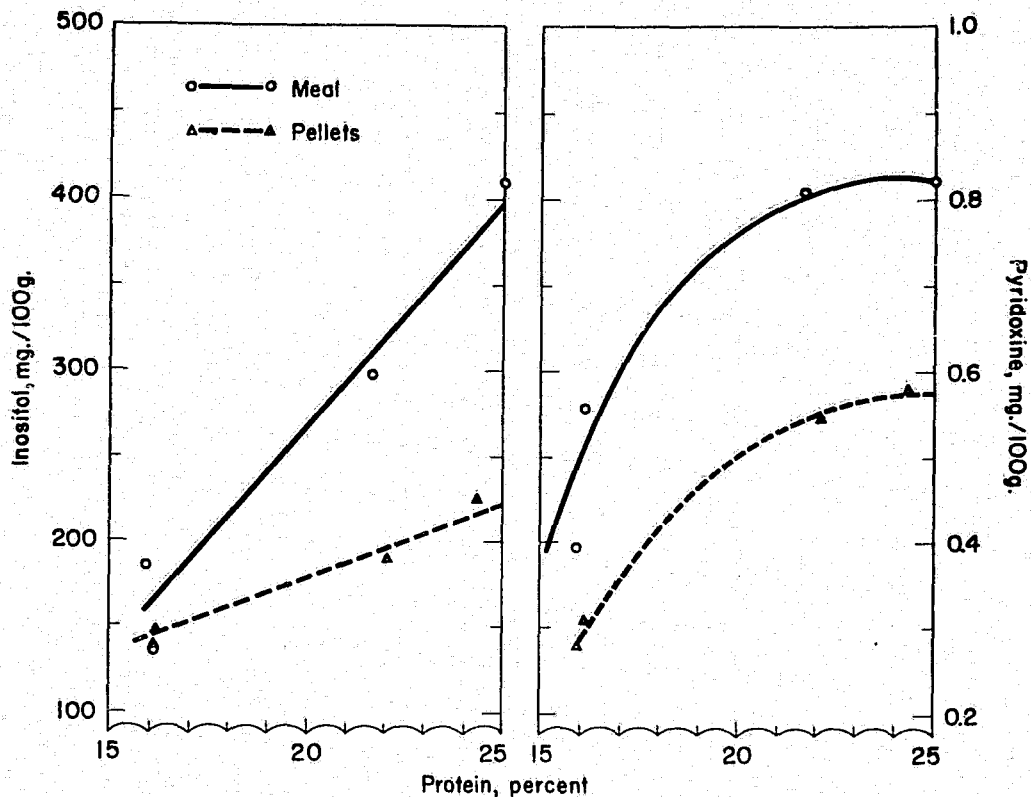


FIGURE 2.—Losses of vitamins inositol and pyridoxine as a result of pelleting and regrinding dehydrated alfalfa meal. Vitamin data are given as milligrams per 100 grams and protein as percentage of moisture- and grit-free original material. Differences between meals and pellets are significant at the 5-percent level.

TABLE 8.—Composition of 12 dehydrated alfalfa meals averaged according to 3 protein levels

Constituents	Protein levels		
	15.5 percent for samples N-1, N-2, C-1, O-1	20.6 percent for samples N-3, C-2, C-4, O-2, O-3	24.8 percent for samples N-4, C-3, O-4
Proximate constituents and organic acids: ¹	Percent	Percent	Percent
Crude fat	2.60	3.40	3.91
Crude fiber	34.8	26.6	18.8
Ash	9.1	0.3	11.1
Sugar, as glucose—reducing	1.18	1.32	1.48
total	3.12	3.23	3.44
Nonvolatile organic acids: ²	2.95	4.25	4.84
Summative constituents: ³			
Azetropes solubles	18.6	20.3	22.7
Oxalate solubles	10.4	24.0	28.8
Total solubles	38.0	45.2	61.5
Doubly extracted residue: ⁴	62.0	54.8	48.5
Protein in residue	10.5	14.3	18.2
Protein-free residue: ⁵	51.5	40.5	30.3
Chlorite holocellulose	52.8	45.6	39.3
Protein in holocellulose	6.2	8.8	11.6
Lignin in holocellulose	3.5	3.0	3.0
Corrected holocellulose	43.1	33.8	24.7
Pentosan in holocellulose	8.1	5.7	3.7
Cellulose: ⁶	35.0	28.1	21.0
Lignin	10.5	7.7	6.2
Lignin as percentage of protein-free residue	20.4	19.0	20.4
Total cell-wall material: ⁴	53.6	41.5	30.9
Vitamin constituents: ⁷	Mg./100 g.	Mg./100 g.	Mg./100 g.
β -carotene: ⁸	9.6	20.3	23.2
Xanthophylls: ⁸	19.3	48.3	50.7
Total tocopherols	15.8	19.1	20.1
Vitamin K	1.61	2.16	1.84
Thiamine	.31	.53	.60
Riboflavin	.82	1.58	1.60
Pantothenic acid	2.69	5.13	4.86
Niacin	3.01	5.04	5.45
Pyridoxine	.67	.90	.91
Inositol	158	216	313
Folic acid	.18	.36	.37
Choline	98	134	169
Betaine	233	255	308

¹ Data as percentage of moisture- and grit-free original material.² Excluding oxalic acid. Calculated, assuming an average equivalent weight of 80 (see text).³ These data calculated by difference.⁴ Total cell-wall material = cellulose + pentosan + lignin (does not include pectin).⁵ Data as milligrams per 100 grams of moisture- and grit-free original material.⁶ Values are low because of delay in assay (see text).

TABLE 9.—Alfalfa as a source of required vitamin factors for poultry

Vitamin factor	Amount required for starting chicks ¹	Percentage of requirement provided by using 5 percent of 20-percent protein alfalfa in the diet
β -carotene	Mg./lb. feed 0.72	640
Choline	600.	5
Vitamin K	.18	270
Riboflavin	1.3	28
Niacin	12.	10
Pantothenic acid	4.2	28
Folic acid	.25	33
Thiamine	.8	16
Pyridoxine	1.3	15

¹ National Research Council, Committee on Animal Nutrition (25).

SUMMARY

Protein content of dehydrated forages was correlated positively with content of other valuable nutrients, and negatively with constituents considered to be of no, or low, nutritive value.

The process of pelleting and regrinding dehydrated alfalfa meal resulted in losses of inositol and pyridoxine vitamins and an apparent increase in ethyl ether extractable material. These changes were not large enough to account for the growth stimulation reported for pelleted forages. This phenomenon may be related to the increase in bulk density resulting from the pelleting process.

When dehydrated forages were subjected to successive extractions with ethanol-benzene azeotrope and dilute ammonium oxalate, the extractions removed 20 to 40 percent of the Kjeldahl nitrogen present in the original material.

Holocellulose, prepared by the acid chlorite method, did not represent total cell-wall carbohydrate material. Residual crude protein and lignin constituted as much as 40 percent of the holocelluloses isolated from dehydrated alfalfa, grass, or grass-legume samples.

Total cell-wall material (cellulose+pentosan+lignin) could be predicted from a determination of the quantity of protein-free, doubly extracted residue in a forage sample. Within 95 percent confidence limits, a 1:1 relationship may be used for total cell-wall material and protein-free, doubly extracted residue with a high degree of accuracy, and for many purposes they may be considered to be equal. This was shown to be true for all types of forage samples analyzed.

The lignin content of a dehydrated alfalfa sample (meal or pellet) could be estimated as 20 percent of the quantity of protein-free, doubly extracted residue determined in that sample. This estimate was also shown to be quite reliable and sufficiently accurate for many purposes. The use of this method would result in a considerable saving in time and labor normally required for direct lignin assays of alfalfa.

It was not possible to determine a general factor for estimating lignin in the grasses and grass-legume mixtures, as only one sample of each species was analyzed. If, however, examination of a larger number of samples demonstrates that lignin represents a constant percentage of the cell-wall material within a species (as was shown with alfalfa), then this same method of lignin estimation will be possible.

Average values were calculated for all constituents determined in the 12 dehydrated alfalfa meals. These averages were grouped according to three protein levels—approximately 15, 20, and 25 percent, corresponding to the most commonly available commercial products.

Dehydrated alfalfa meal containing 20 percent protein and used at a level of 5 percent of a poultry ration contributes significant quantities of many nutrients essential to proper poultry growth.

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