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Received for review January 17, 1957. Accepted April 9, 1957.

DEHYDRATION TEMPERATURE EFFECTS

Effect of Temperature of Dehydration on Proteins of Alfalfa

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The effect of temperature of dehydration on the proteins of alfalfa was investigated. There was no difference in the total nitrogen contents of meals dehydrated at 50° C. and dehydrated commercially. The low temperature meal, as opposed to the high temperature meal, contained more α-amino nitrogen and less protein nitrogen, contained some water-soluble protein, and possessed proteolytic activity. Digestion by pepsin, trypsin, and erepsin, or by high levels of pancreatin, showed no differences in the digestibilities of the meals. Suboptimum quantities of pancreatin liberated α-amino nitrogen at a greater rate from low temperature meal than from commercially dehydrated meal. Extracts of the meals inhibited the action of trypsin on casein and the inhibition was not decreased by autoclaving the extracts.

About a million tons of dehydrated alfalfa meal are produced each year. Dijkstra (*6*) reported that the commercial dehydration of grass reduced the in vivo digestibility of its protein. Brew (*5*) showed that the temperatures used in dehydrating alfalfa commercially were sufficient to alter the solubility of the proteins. This is a report of a further investigation of the protein changes caused by commercial dehydration of alfalfa meal and their possible effect on the nutritive value of the meal.

Experimental

Samples. Three alfalfa meals were prepared by subjecting freshly chopped, first-cutting alfalfa to different heat treatments. One portion was dried at 50° C. in a circulating air oven. A second portion was dehydrated in a commercial dehydrator operating with an air inlet temperature of approximately 900° C. and an air outlet temperature of about 175° C. High temperature–pelleted meal was prepared by passing a portion of this meal through a California pelleting machine and regrinding it to pass through a 20-mesh screen. The latter sample was prepared because the meal is subjected to additional heat in the pelleting operation.

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Nitrogen Fractions. The effect of the heat treatments on certain nitrogen fractions was studied. The total nitrogen content of each of the three meals was determined by the Kjeldahl procedure (*1*). To measure the water-soluble nitrogen, 5 grams of meal were extracted with 200 ml. of cold water for 10 minutes in a Waring Blendor, the extract was filtered, and a 50-ml. aliquot was analyzed for nitrogen as before. A second portion of the filtered extract was adjusted to pH 4.5, heated to boiling, and filtered again. Fifty milliliters of this solution were analyzed for nitrogen also. The difference between the nitrogen values of the two aliquots represents soluble protein nitrogen. The remainder of the heated solution was neutralized and was analyzed for α-amino nitrogen by a flame photometric procedure (*2*).

Table I shows that there was little difference in the total nitrogen content of the three meals. However, differences between the low and high temperature meals were noted in the nitrogen breakdown. The conversion of soluble to insoluble protein by the heat of the commercial dehydration process confirms the qualitative data of Brew (*5*). The higher α-amino nitrogen and lower protein nitrogen of the low temperature meal indicate that proteases were active during low temperature drying.

Proteolytic Activity of Meals. The meals were tested for the presence of proteolytic activity by observing their effect on casein solutions. Ten milliliters of a 1% casein solution, 20 ml. of water, and 1 or 2 crystals of thymol were placed in each of two beakers. One gram of low temperature meal was added to one of the beakers and 1 gram of high temperature meal to the other.

Table I. Quantity of Nitrogen in Some Fractions of Alfalfa Meals Subjected to Different Heat Treatments

Meal[a]	Total Nitrogen (A)	Water-Soluble Nitrogen (B)	Nonprotein Nitrogen (C)	Soluble Protein Nitrogen (B − C)	Total Protein Nitrogen (A − C)	α-Amino Nitrogen (D)	Unidentified Nitrogen (C − D)
	Mg./G.						
1	33.6	11.4	9.7	1.7	23.9	3.7	6.0
2	32.8	5.2	5.2	0.0	27.6	2.7	2.5
3	32.9	5.6	5.4	0.2	27.5	2.6	2.8

[a] 1, low temperature meal; 2, high temperature meal; 3, high temperature–pelleted meal.

The contents of the beakers were stirred for 5 minutes and the pH was adjusted to 5.5 with glacial acetic acid. The beakers were covered with watch glasses and placed in a water bath at 37° C. for 12 hours.

Similar digestions were carried out at pH 7.0 and 8.4. At the end of the digestion period, the samples were adjusted to pH 4.5 with glacial acetic acid and boiled for 3 minutes on a hot plate. This inactivated the enzymes, if present, and precipitated undigested protein. The hydrolyzates were cooled, made to a volume of 50 ml., and filtered. The filtrates were neutralized and analyzed for α-amino nitrogen by the flame photometric procedure. Six other digestions were prepared as above, except that hydrolysis was stopped at zero time. The increase in α-amino nitrogen during hydrolysis was obtained by subtracting the α-amino nitrogen found in the zero-time hydrolyzates from that found in the corresponding 12-hour hydrolyzates. The results are presented in Table II.

Table II. Release of α-Amino Nitrogen from Casein Incubated with Alfalfa Meals

Meal	pH 5.5	pH 7.0	pH 8.4
	Mg./L.		
Low temperature	67.0	55.0	22.5
High temperature	0.5	0.5	0.5

The greatest proteolytic activity was obtained at pH 5.5, but there still was good activity at pH 7.0. Other workers have shown that the optimum pH of many plant proteases is near or slightly below 7 (*3*). This experiment shows that sufficient heat is used in the commercial dehydration of alfalfa to inactivate the proteolytic enzymes of the meal.

Hydrolysis by Pepsin, Trypsin, and Erepsin. Evans (*7*) showed that autoclaving soybean meal at high temperatures reduced in vitro digestibility of the soybean protein by pepsin, trypsin, or erepsin when the enzymes were used alone or in combination. The alfalfa meals in this study were subjected to the action of the above enzymes by a modification of the method of Evans.

One gram of meal and a few crystals of thymol were placed in a 150-ml. beaker. A pepsin solution was prepared containing 2 mg. of enzyme (1 to 10,000) per ml. of water, and enough solution was added to the beaker to supply 1 mg. of enzyme per mg. of protein nitrogen. The protein nitrogen values used were obtained from the nitrogen fractionation studies—e.g., low temperature meal, 23.9; commercially dehydrated meal, 27.6; and high temperature–pelleted meal, 27.5 mg. per gram of meal. Twenty-five milliliters of 0.1*N* hydrochloric acid were added to the beaker. The beaker then was covered with a watch glass and placed in a water bath maintained at 37° C.

After 12 hours, either the digestion was stopped and the hydrolyzate was analyzed for α-amino nitrogen, or the digestion was continued with trypsin. When the digestion was continued, 20 ml. of phosphate buffer (*2*) were added to the hydrolyzate and the pH was adjusted to 8.4 with 5% sodium hydroxide. Trypsin (1 to 300) was added to the hydrolyzate in the same form and quantity as pepsin had been added previously. The digestion was continued at 37° C. for 12 hours. At the end of this period, either the hydrolyzate was analyzed for α-amino nitrogen or the digestion was continued with erepsin, added in the same manner and at the same rate as pepsin and trypsin had been added. The digestion was continued at 37° C. for 12 hours, after which the hydrolyzate was analyzed for α-amino nitrogen. Appropriate blank digestions were carried through the procedure, using enzymes which had been inactivated by heat. Also, both active and inactive enzymes were carried through the digestion procedure without alfalfa in the digestion mixture.

The amount of α-amino nitrogen liberated by an enzyme or combination of enzymes was found by subtracting the α-amino nitrogen in a hydrolyzate, which had contained heated enzyme, from that in a hydrolyzate which had contained active enzyme. These calculated values are shown in Table III on the line entitled "none" for the hydrolyzates which contained enzymes but no meal, and on the lines entitled "apparent" for the hydrolyzates which contained both enzymes and meal.

α-Amino nitrogen was liberated in the hydrolyzates which contained active enzymes but no meal. Hence, to find the amount of α-amino nitrogen liberated from the protein of the meals only, it was necessary to correct the apparent amount liberated from the meals for the amount liberated from the enzymes themselves. These corrected values also are shown in Table III.

Trypsin extended the digestion beyond that liberated by pepsin alone and, although erepsin apparently increased the liberation of α-amino nitrogen beyond that obtained by pepsin and trypsin, the corrected values of the enzyme combinations were essentially the same.

The use of greater quantities of these enzymes did not increase the digestion of the meals, and with longer periods of hydrolysis, trouble was encountered because of fermentation in the digestions. Hydrolysis with 8*N* hydrochloric acid for 8 hours liberated 16.8 mg. of amino nitrogen from low temperature meal, 17.8 mg. from high temperature meal, and 17.9 mg. from high temperature–pelleted meal. The latter values may be less than the potential α-amino nitrogen of the protein because of losses of α-amino nitrogen in humin formation, but they are greater than the maximum values obtained by the enzymic digestions. Hence, the proteins of alfalfa meal were not hydrolyzed completely by these enzymes under the conditions employed in this study.

Hydrolysis by Pancreatin. Melnick and Oser (*10*) showed that when the biological value of a protein was altered by a heat treatment, the rate of liberation of α-amino nitrogen from the protein by suboptimum quantities of pancreatin was altered also. The alfalfa meals used in this investigation were subjected to the action of pancreatin (3 × United States Pharmacopeia) by a modification of the procedure of Melnick and Oser (*2*, *10*). Two levels of pancreatin were employed, 0.2 and 1.0 mg. of enzyme per mg. of protein nitrogen. One gram of meal and one or two crystals of thymol were placed in a 150-ml. beaker. Twenty-five milliliters of water and 20 ml. of phosphate buffer (pH 8.4), both prewarmed to 37° C., were added to the beaker. Then enough of the pancreatin suspension, also prewarmed, was added to supply the level of enzyme desired. The contents of the beaker were stirred

Table III. Liberation of α-Amino Nitrogen from Alfalfa Meals by Pepsin, Trypsin, and Erepsin

Meal	Enzymes Used, Mg./G. Pepsin	Pepsin + trypsin	Pepsin + trypsin + erepsin
None	0.1	0.6	2.4
Low temperature			
Apparent	4.0	11.2	13.4
Corrected	3.9	10.6	11.0
High temperature			
Apparent	4.2	11.6	13.2
Corrected	4.1	11.0	10.8
High temperature–pelleted			
Apparent	3.9	11.4	13.6
Corrected	3.8	10.8	11.2

for 5 minutes, the pH was adjusted to 8.4 with sodium hydroxide, and the beaker was placed in a water bath at 37° C. The digestions were carried out for 0, 3, 6, 12, and 24 hours. Appropriate blanks using heated enzymes were carried through the procedure also. The resulting hydrolyzates were analyzed for α-amino nitrogen, and the amount liberated from the meals was plotted against the hydrolysis time in hours to obtain the curves shown in Figure 1.

With suboptimum quantities of pancreatin there seems to be a difference in the case of hydrolysis of the proteins of the meals. However, Melnick and Oser (*10*) reported that the native proteolytic enzymes of raw soybean meal work synergistically with pancreatin. Although no proteolytic activity was observed in the digestions containing low temperature meal and heated pancreatin, it is possible that the native proteolytic enzymes of the meal could work synergistically with active pancreatin to increase rate of liberation of α-amino nitrogen observed with this meal.

Sumner and Somers (*11*) pointed out that partially hydrolyzed proteins are more easily digested by trypsin than are native proteins. Perhaps one action of the native proteolytic enzymes is to cleave the protein into large fragments. Such action would liberate relatively small quantities of α-amino nitrogen, but might render the partially hydrolyzed protein more susceptible to digestion by trypsin. The reason for the greater rate of digestion obtained with high temperature–pelleted meal as compared with high temperature meal which was not pelleted is not known. It might have been due to the additional heat supplied to the meal during the pelleting operation, or perhaps a finer grind was obtained with the pelleted meal than with the high temperature meal, making the protein of the meal more readily available to the enzyme.

Trypsin Inhibition Studies. Kendall (*8*) reported that the in vitro digestion of casein by trypsin is inhibited by extracts of the fresh forage of alfalfa, ladino clover, and soybeans. It was of interest to determine if this inhibitory effect could be observed with extracts of dehydrated alfalfa meal, and to study the effect of the temperature of dehydration on the magnitude of the inhibition. Twenty-five grams of meal were extracted with 75 ml. of water. The extract was expressed through cloth and centrifuged at 2000 r.p.m. The supernatant liquid was divided into two portions. One portion was used in the digestions without further treatment, while the other portion was autoclaved at 15 pounds' pressure for 15 minutes.

For the digestions, 20 ml. of a 1% solution of casein in phosphate buffer (pH 8.4), 70 ml. of water, and either 6 ml. of water or 6 ml. of the extract were placed in an 8-ounce screw-cap, wide-mouthed jar, and the contents of the jar were warmed to 37° C. Four milliliters of a trypsin solution containing 37.5 mg. of enzyme per 100 ml. of water were added to the jar and the system was incubated at 37° C. At the end of 4 hours, the digestate was acidified to pH 4.5 and heated almost to boiling for 1 minute. After the hydrolyzate had cooled, it was filtered and analyzed for α-amino nitrogen by the flame photometric procedure. Blank digestions were carried out using trypsin, which had been inactivated by heat. The liberation of α-amino nitrogen was calculated by subtracting the amount found in a digestion which had contained inactivated enzyme from the amount in a corresponding digestion which had contained active enzyme (Table IV).

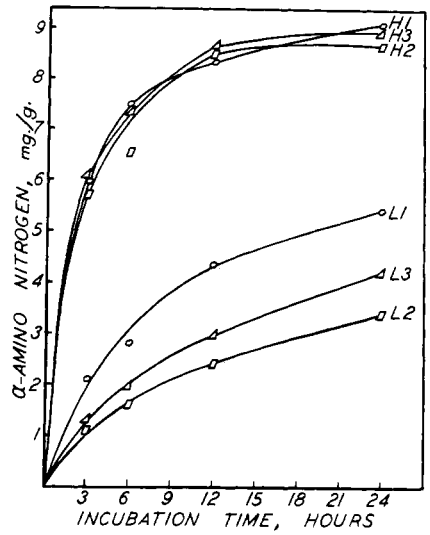

Figure 1. Liberation of α-amino nitrogen from three alfalfa meals by two levels of pancreatin

H, high level; L, low level; 1, low temperature meal; 2 high temperature meal; and 3, high temperature–pelleted meal

Table IV. Effect of Aqueous Extracts of Alfalfa Meal on Liberation of α-Amino Nitrogen from Casein by Trypsin

Extract	α-Amino Nitrogen, Mg./L.
None	58
Low temperature meal	
Unautoclaved	38
Autoclaved	38
High temperature meal	
Unautoclaved	38
Autoclaved	36
High temperature–pelleted meal	
Unautoclaved	37
Autoclaved	37

There was a decrease in the amount of α-amino nitrogen liberated from the digestions, which contained extracts of the alfalfa meals (Table IV). Previous heat treatments caused no difference in the extent of inhibition observed. The autoclaved extracts showed the same degree of inhibition as the unautoclaved extracts.

It was thought that the observed decrease in α-amino nitrogen might be due to adsorption of the liberated amino acids on the colloidal materials present in the meal extracts. This possibility was investigated by performing digestions of casein by trypsin in which the meal extract was added either at the beginning or end of the hydrolysis period. Less α-amino nitrogen was found in the hydrolyzates which contained the extract at the beginning of the digestion period. Hence, it was concluded that the alfalfa meal extract inhibited the action of trypsin.

Discussion

The differences found in the nitrogen fractions of the meals may have some nutritional significance. The greater amount of water-soluble nitrogen found in the low temperature meal may make more nitrogen more readily available to the animal. Also, the native proteolytic enzymes of low temperature meal probably would be active in the alimentary tract of an animal, and perhaps would work synergistically with the digestive enzymes of the animal to produce a greater degree of protein digestion than with high temperature meal.

Although only about one third of the protein nitrogen of the meals was liberated as α-amino nitrogen by the combined action of pepsin, trypsin, and erepsin, the extent of hydrolysis was of the same magnitude as obtained by Evans (*7*) on soybean meal. The digestive system of an animal probably would be more effective in digesting the proteins than were these enzymes under in vitro conditions.

The pancreatic digestion studies indicate the importance of digesting protein with the proper level of enzyme in order to demonstrate differences in protein digestibility. With the higher level of pancreatin, the differences in digestibility of the proteins of the meals were obscured. The lower rate of liberation of α-amino nitrogen from the proteins of high temperature meal by suboptimum quantities of pancreatin indicates that this meal may be inferior to low temperature meal as a source of protein for animals (*10*). Low temperature meal contained less protein nitrogen than high temperature meal because of the enzymic breakdown of protein during drying, and if the digestions had been performed with a constant amount of protein instead of a constant amount of meal, the differences in rates of digestion between the low and high temperature meals would have been about 15% greater.

The nature of the trypsin inhibition is

unknown. It seems that the inhibition is not due to a heat-labile substance. Kendall and Touchberry (*9*) showed that the inhibition of trypsin by soybean forage extracts increases as the seed begins to form. Borchers and Ackerson (*4*) reported the presence of a trypsin inhibitor in the seed of alfalfa; therefore, it seems possible that the inhibitory effect may be one of the causes of slow growth, obtained when high levels of alfalfa meal are included in chick rations.

A different result may be obtained from in vitro studies than under in vivo conditions. Hence, in vivo techniques will be required before the final answer to this problem is obtained.

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Received for review November 14, 1956. Accepted February 25, 1957. Division of Agricultural and Food Chemistry, ACS, 131st Meeting, Miami, Fla., April 1957. Contribution No. 547, Department of Chemistry.

SILAGE EVALUATION

Polyunsaturated Fatty Acids in Legume-Grass Silage

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The effects of time after ensiling, presence or absence of preservative, and of crop ensiled on the linoleic, linolenic, and total long-chain fatty acids in legume-grass silage were studied employing laboratory silos (glass jars), concrete miniature silos, and a bunker-type silo. There appeared to be no major differences between silages with and without added preservatives as regards the polyunsaturated and total long-chain fatty acids in silages made in the three types of silos. The silage fermentation process caused no major change in the amount of polyunsaturated fatty acid in the total dry matter. However, the percentage of linoleic acid in the total fatty acid of silages was distinctly lower than that in the original forage at the time of ensiling.

THE HIGHLY UNSATURATED FATTY acids are major constituents of the saponifiable fraction of lipides extracted from green plants (*8, 10, 11*). Moreover, the presence of polyunsaturated fatty acids in alfalfa leaf meal (*6*) and in buckwheat leaf meal (*7*) has been established. As linoleic and linolenic acids are the principal polyunsaturated fatty acids in these green plants and meals, the consumption of such materials helps provide the fatty acids considered to be essential in the nutrition of farm animals. Increasing use of silages made from legumes and grasses emphasizes the need for data on the composition of this type of livestock feed. The paucity of information concerning the levels of the polyunsaturated fatty acids remaining in the silage after the fermentation has taken place led to the present investigation.

Experimental

Three types of silos, as described below, were employed in this study.

One-gallon glass jars were used as miniature laboratory silos for comparing silages treated with sodium nitrite (8 pounds per ton), calcium acetate (8 pounds per ton), and dried beet pulp (160 pounds per ton) with silage to which no preservative was added. Samples of freshly cut legume-grass forage (mostly clover) were mixed with appropriate preservatives and packed into jars which were then sealed with screw-cap lids and paraffin. Each treatment was replicated eight times and the bottles were stored at 75° to 80° F. Samples were taken for analysis at the time of ensiling and at 1, 4, 7, 14, 21, 28, 56, and 84 days thereafter.

Miniature concrete silos, 6 feet high and 2 feet in diameter, were used to study the effects of various silage preservatives added to chopped, nonwilted, legume-grass mixture. Sodium metabisulfite (8 pounds per ton), calcium formate (20 pounds per ton), cane sugar (10, 20, and 30 pounds per ton), dried beet pulp (160 pounds per ton), and ammonium acetate (8 pounds per ton) were tested in this experiment. Each preservative was employed in at least two silos and, for each trial, duplicate silos were filled with forage to which no preservative was added. The forage in each silo was tramped thoroughly during the filling operation and subsequently at daily intervals for 3 days, after which each silo was sealed with a plastic cover. Samples for analysis were taken at the time of filling and when the silos were emptied at times ranging from 4 to 10 weeks after ensiling.

A bunker-type silo (24 × 100 foot concrete slab with a 4-foot wall along one side) was filled with approximately 450 tons of chopped, nonwilted forage (mostly alfalfa with some brome grass), half of which was treated with ground corn (100 pounds per ton) as a preservative. The forage was packed thoroughly with a tractor during the filling operation and for an hour or more for each of 6 days after the silo was filled. Samples for analysis were taken at the time of ensiling and from various areas of the stack 22 and 31 weeks later.

Each sample taken was 1 to 2 pounds of forage or silage and was a composite representing either an entire miniature