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Bachelor of Science

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Stillwater, Oklahoma

1951

Submitted to the Faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
in Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE
1953

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ACKNOWLEDGMENT

The author wishes to acknowledge his appreciation to Doctor Robert MacVicar for his guidance and assistance throughout the course of these studies. Thanks are also due to Doctor H. F. Murphy of the Department of Agronomy and to Mr. Charles Chappel of the Department of Animal Husbandry for providing experimental materials used in these studies.

The author is further indebted to the Department of Agricultural Chemistry Research and the Oklahoma Agricultural Experiment Station for the use of their laboratories and experimental facilities and for financial assistance during the course of these studies.

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I. RIBOFLAVIN, NICOTINIC ACID, AND PANTOTHENIC ACID IN RUMEN CONTENTS OF SHEEP

INTRODUCTION

Many thousands of pounds of vitamins are discarded yearly by the packing industries in disposing of the contents of the rumen from the cattle and sheep. Until the last few years, no attempt has been made to process the rumen contents to recover any of these valuable nutrients. The recovery of a portion of the vitamins present in the rumen contents might provide a means of economically disposing of this waste material as well as produce a product of considerable value as a vitamin supplement for swine, chickens, and other non-ruminants.

The feasibility of processing rumen contents to recover the vitamins would depend on whether the quantity of vitamins was sufficient to make it profitable. Since it has been observed that the vitamin concentration of rumen contents varies greatly depending on the type and quality of ration fed, this study has been made to determine the effect of differences in (1) the quality of roughage fed, (2) the availability of the carbohydrate fed, and (3) the inorganic matter fed on the riboflavin, nicotinic acid, and pantothenic acid content of the whole rumen material.

REVIEW OF LITERATURE

The suggestion that the bacteria of the rumen might synthesize vitamins was probably first presented by Theiler, Green, and Viljoen (38) in 1915. They stated, "We think it is at least possible that the vitamin requirements of cattle are so low that they may even be covered indirectly by synthesis carried out by the extensive bacterial flora of the intestine." In 1923 using Bacillus vulgatus, an organism found in the rumen, in in vitro studies Sheunert and Shieblich (31) demonstrated that these organisms could synthesize vitamin B.

In 1928 Bechdel et al. (4), using a cow with a permanent fistula for sampling the rumen contents, fed rats an extract prepared from the rumen contents and this extract proved to be a potent source of vitamin B, although the ration fed the cow was practically devoid of the vitamin B complex. In the same study they found that a bacteria which they designated as of the genus Flavobacterium, which predominates in the rumen, also produced the vitamin B complex when grown in vitro.

After these preliminary qualitative investigations were carried out, McElroy and Goss (24, 25, 26, 27, 28) in a series of quantitative studies demonstrated that thiamin, riboflavin, pantothenic acid, and pyridoxine were synthesized in the rumen. Their studies were carried out using both sheep and cattle fed a ration practically devoid of the vitamin B complex. They found an increase of riboflavin from 0.3 micrograms per gram in the ration to 33 micrograms per gram in the dried rumen contents, an 8-10 fold increase in pyridoxine, a 25-30 fold increase in pantothenic acid, and a slight increase for thiamin. Wegner and co-workers (41) in experiments carried on at the same time as those of the above group, were also able to show that the bovine species is able to synthesize significant amounts of thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid and biotin when fed a ration low in these compounds. Hunt and associates (14) also found that riboflavin and thiamin are synthesized in the rumen, but that the thiamin is either absorbed or destroyed quickly.

Rumen contents or some concentrate made from rumen contents might be used for a supplement for chicks and other non-ruminants. Booth and Hart (6) proposed a method for making a concentrate in which they heated rumen contents to 194° F., pressed out the fluid, and evaporated the solution to dryness at low temperature obtaining an extract containing 17 to 20 micrograms of riboflavin per gram. Hammond (11) used dried rumen contents as a substitute for alfalfa leaf meal in a chick ration and found it to be as good or better than the alfalfa leaf meal as a vitamin source. Frey and Pratzer (9) also found that dried rumen contents would furnish most of the vitamin requirements for chicks.

That the type and quality of ration fed affects the production of vitamins in the rumen is suggested by the statement of Hunt and associates (13), "It is also apparent that the kind and state of the feed consumed are factors in the synthesis of riboflavin in the rumen." Their results indicated that the higher the carbohydrate content of the rations the greater the riboflavin production in the rumen. Lardinois et al. (16) found that if a readily available carbohydrate were present there was an increased synthesis of nicotinic acid, biotin, riboflavin, and pantothenic acid in the rumen. That mineral constituents also affect the vitamin synthesis was demonstrated by Hale and co-workers (10) using chicks to assay the rumen contents of cobalt deficient and cobalt supplemented sheep for vitamin B_{12}. The chicks on the ration containing rumen contents from the cobalt supplemented sheep grew much faster than those on the ration containing the rumen contents of the cobalt deficient sheep.

MATERIALS AND METHODS

Source of Material. Sixteen sheep were paired according to size and weight by Mr. Chappel of the Animal Husbandry Department. The pairs were fed eight different rations of composition as shown in Table I for thirty days to bring the contents of the rumen into equilibrium with the ration being fed. The sheep fed rations 1 through 6 were provided with salt *ad libitum*. The sheep fed rations 7 and 8 were allowed only the minerals included in their respective rations. At the end of this equilibrating period the sheep were sacrificed four at a time on successive days.

Collection and Treatment of Materials. The rumen and reticulum contents were removed from the sheep as quickly as possible and acidified to minimize vitamin destruction, then the contents were placed in shallow pans and autoclaved for five minutes at fifteen pounds pressure to stop bacterial action. The contents were then dried in a forced draft oven at 80° C. after which they were ground in a Willey Mill, placed in sample bottles, and stored until they were analyzed for riboflavin, nicotinic acid, and pantothenic acid.

Analytical Methods. The vitamin contents were determined by microbiological assay on acid or enzyme digests of homogeneous samples of the rumen contents. Hydrolysates for nicotinic acid analysis were prepared by heating 2.5 grams of dry matter with 50 ml. of 0.1 N sulfuric acid in an autoclave for 15 minutes at 15 pounds pressure. The samples were then neutralized, diluted to a volume of 100 ml. and filtered. The filtrates were analyzed for nicotinic acid using Lactobacillus arabinosis 17-5 essentially according to the procedure of Snell and Wright (37). Hydrolysates for riboflavin determination were prepared by heating 2.5 grams of dry material with 50 ml. of 0.1 N Hydrochloric acid in an autoclave for 20 minutes at 15 pounds pressure. These samples were then neutralized using 0.5 N potassium hydroxide to prevent sodium chloride toxicity (22), diluted to a volume of 100 ml., and filtered. The filtrates were analyzed for riboflavin using Lactobacillus casei and essentially the procedure of Snell and Strong (36). For the determination of pantothenic acid the digestion procedure used was that of Ives and Strong (15) employing "Mylase P." One gram of dry material was suspended in 50 ml. of distilled water, autoclaved for 15 minutes at 15 pounds pressure, cooled to room temperature, and 0.1 gram of "Mylase P" added. The solution was buffered with 2.5 ml. of 2.5 M sodium acetate and the pH adjusted to 4.8 using a Beckman pH meter. The mixture was then layered with toluene, corked and incubated for 24 hours at 50° C. At the end of the incubation period the samples were diluted to 100 ml., readjusted to a pH of 4.8, and filtered. The filtrates were then analyzed for pantothenic acid using Lactobacillus casei and the media and procedure of Barton - Wright (3).

It is recognized that the currently available methods of hydrolysis and digestion, such as were employed in this study, result in lower than the true values due to partial destruction during the drying process and in the case of pantothenic acid not all of it being freed by the digestion procedure. Values presented in Tables II, III, and IV should, therefore, be regarded as minimal particularly in the case of riboflavin and pantothenic acid. Such minimum values, nevertheless, are useful in evaluation of the effects of different factors on the production of the vitamins by the bacteria of the rumen. The values given are the averages of at least two assays on a single hydrolysate for nicotinic acid, and averages of assays from two separate hydrolysates and digestions for riboflavin and pantothenic acid.

TABLE I

COMPONENTS OF RATIONS CONSUMED BY SHEEP

Feed	Ration Number (amount fed in grams air dry basis)							
	1	2	3	4	5	6	7	8
Alfalfa Hay					360			
Prairie Hay	600	360	360					
Sorghum Silage				360				
Corn		422	320	350	420	720	177	177
Soybean Meal			120	90	20	80		
Urea		18						
Corn Cobs							318	318
Corn Gluten Meal							141	141
Corn Syrup							49	49
Corn Oil							21	21
$CaHPO_4$							6	6
Alfalfa Ash								42
Total Amount Per Day	600	800	800	800	800	800	712	754

RESULTS AND DISCUSSION

The vitamin content (riboflavin, nicotinic acid, and pantothenic acid) of rumen contents and the rations fed are presented in Tables II through V. The vitamin synthesis in the rumen is shown by the percentage increase of the amount of vitamin in the rumen contents above the amount calculated to be present in the ration based upon the analysis of the ration components.

Riboflavin (Table II). The addition of carbohydrate and a nitrogen source (Ration 1 vs. 2) more than tripled the synthesis of riboflavin by rumen microorganisms. The concentration was 6.2 micrograms per gram in the rumen contents of the animal fed ration 1, an increase of 88% above the amount present in the prairie hay. Rumen contents of the animals fed ration 2 contained 8.6 micrograms per gram, an increase of 290%. Altering the source of nitrogen (Ration 2 vs. 3) did not change the amount of riboflavin in the rumen contents appreciably, 8.6 micrograms per gram as compared with 9.5 micrograms per gram (290 vs. 296% increase). Comparing the effect of different types of roughage (Rations 3, 4, and 5) it is seen that the silage favors the synthesis of riboflavin, the content increasing 327% on the silage ration, 296% on the prairie hay ration, and 125% on the alfalfa ration. Absolute concentrations for these treatments are 9.5, 13.7, and 11.7 micrograms per gram respectively. Thus, although the amount synthesized varied widely, the absolute amount in the rumen contents was shifted within relatively narrow limits. Rations 1, 3, and 6 demonstrate that a more readily available carbohydrate greatly increases the synthesis of riboflavin with percentage increases of 88, 296, and 560% respectively; however, as above, the absolute values varied relatively less, 6.2, 9.5, and 9.9 micrograms per gram. It would appear that the provision of an adequate supply of energy and protein tends to stabilize the riboflavin concentration of the rumen contents. Influence of inorganic nutrients is demonstrated by the comparison of rations 7 and 8. The addition of alfalfa ash caused an increase in the amount of riboflavin in the rumen contents from 6.4 micrograms per gram (Ration 7) to 8.2 micrograms per gram. This represented a 256% increase in ration 7 and a 356% increase in ration 8.

Nicotinic Acid (Table III). The concentration of nicotinic acid in rumen contents was increased from 51.4 to 84.0 micrograms per gram by the addition of carbohydrate and nitrogen to a prairie hay ration (Ration 1 vs. 2). The percentage increases were 254 and 453 respectively above the amount of nicotinic acid present in the ration. Substitution of soybean meal for urea as the source of nitrogen (Ration 2 vs. 3) increased the concentration of nicotinic acid from 84.0 to 100.4 micrograms per gram and the synthesis from 452 to 513% above that in the ration consumed. It is seen by comparison of rations 3, 4, and 5 that silage favored the synthesis of nicotinic acid as it did riboflavin. Absolute concentrations were 100.4, 148.6, and 138.8 micrograms per gram for the respective treatments with percentage increases of 513, 604, and 493. That a more readily available source of carbohydrate increased the nicotinic acid concentration of rumen contents was demonstrated by the results found with rations 1, 3, and 6. The absolute concentrations were 51.4, 100.4, and 108.4 micrograms per gram respectively with percentage increases of 245, 513, and 542. The tendency for the concentration of

TABLE II

RIBOFLAVIN CONTENT OF RATIONS CONSUMED AND OF RUMEN CONTENTS OF SHEEP

Ration Number	Ration	Sheep Number	Present in Ration Micrograms/gm. Dry Matter	Present in Rumen Contents Micrograms/gm. Dry Matter	Percentage Increase
1	Prairie Hay	2	3.3	6.3	91
		23		6.1	85
				Ave. 6.2	Ave. 88
2	Prairie Hay, Corn, Urea	5	2.2	8.2	273
		11		8.9	306
				8.6	290
3	Prairie Hay, Corn, Soybean Meal	6	2.4	9.0	275
		18		10.0	317
				9.5	296
4	Silage, Corn, Soybean Meal	7	3.2	15.1	372
		12_2		12.2	281
				13.7	327
5	Alfalfa, Corn, Soybean Meal	10	5.2	12.1	133
		29		11.3	117
				11.7	125
6	Corn, Soybean Meal	4	1.5	9.1	507
		14		10.7	613
				9.9	560
7	Corn Cob Basal	8	1.8	6.3	250
		12_1		6.5	261
				6.4	256
8	Corn Cob Basal + Alfalfa Ash	1	1.8	7.0	289
		13		9.4	422
				8.2	356

nicotinic acid to be stabilized by improved nutrition was not as great as was the case with riboflavin. Good quality roughages, silage and alfalfa, definitely favored synthesis of nicotinic acid and resulted in a rumen contents which contained more of this vitamin than other rations. The addition of alfalfa ash to the corn cob basal ration (Ration 7 vs. 8) shows the influence of inorganic constituents on the synthesis of nicotinic acid. The percentage increases were 267 and 367 for rations 7 and 8 respectively with absolute amounts of 68.2 and 86.9 micrograms per gram. Addition of this mineral supplement improved nicotinic acid synthesis less strikingly than it affected riboflavin formation.

Pantothenic Acid (Table IV). The addition of carbohydrate and nitrogen (Ration 1 vs. 2) brought about a change from an apparent loss of pantothenic acid to an increase. Absolute concentrations were 8.3 micrograms per gram in the rumen contents of the sheep fed prairie hay alone and 14.3 micrograms per gram in the rumen contents of the sheep fed corn and urea in addition to prairie hay, an increase of 78% above that in the ration consumed. Changing the source of nitrogen (Ration 2 vs. 3) did not change the amount of pantothenic acid in the rumen contents appreciably, 14.3 and 17.5 micrograms per gram respectively (78 vs. 84% increase). By comparing the influence of different types of roughage (Rations 3, 4, and 5) it is seen that there is little effect on the concentration of pantothenic acid. Absolute concentrations for these treatments were 17.5, 16.4, and 22.6 micrograms per gram respectively with percentage increases of 84, 8, and 37. It is seen, that as in the case of riboflavin, the pantothenic acid concentration of rumen contents tends to be stabilized by the provision of energy and protein. A more readily available carbohydrate, (Ration 3 and 6) greatly increases

TABLE III

NICOTINIC ACID CONTENT OF RATIONS CONSUMED AND OF RUMEN CONTENTS OF SHEEP

Ration Number	Ration	Sheep Number	Present in Ration Micr... Microgms/gm. Dry Matter	Present in Rumen Contents Microgms/gm. Dry Matter	Percentage Increase
1	Prairie Hay	2	14.5	50.2	246
		23		52.5	262
				Ave. 51.4	Ave. 254
2	Prairie Hay, Corn, Urea	5	15.2	77.7	411
		11		90.3	494
				84.0	453
3	Prairie Hay, Corn, Soybean Meal	6	16.4	108.1	559
		18		92.8	466
				100.4	513
4	Silage, Corn, Soybean Meal	7	21.1	151.6	618
		12_2		145.5	590
				148.6	604
5	Alfalfa, Corn, Soybean Meal	10	23.4	139.9	498
		29		137.6	488
				138.8	493
6	Corn, Soybean Meal	4	16.9	120.0	610
		14		96.8	473
				108.4	542
7	Corn Cob Basal	8	18.6	69.0	271
		12_1		67.4	262
				68.2	267
8	Corn Cob Basal + Alfalfa Ash	1	18.6	78.6	323
		13		95.2	412
				86.9	367

the concentration of pantothenic acid in the rumen contents giving values of 17.5 and 30.1 micrograms per gram. These compared with 8.3 in the rumen contents of animals receiving prairie hay alone. The all-concentrate ration (Ration 6) highly favored the synthesis of pantothenic acid resulting in the highest values found in this study. The addition of alfalfa ash did not effect the pantothenic acid content to the extent it did riboflavin and nicotinic acid, but there was an increase from 16.4 to 18.7 micrograms per gram in the rumen contents of the sheep fed rations 7 and 8 respectively with percentage increases of 215 and 260.

Although the constituents of the ration did effect the B vitamin composition of the rumen contents of sheep, it is doubtful whether the amounts found even under the most favorable circumstances would be sufficient to make the processing of rumen contents profitable. The average amounts for all various rations were 9.3 micrograms of riboflavin, 98.4 micrograms of nicotinic acid, and 18.0 micrograms of pantothenic acid. These concentrations, though too low to justify separation for their own value, might, however, contribute toward a partial recovery of the cost of disposing of rumen contents. With increasingly severe restrictions on stream pollution, the effluent from packing houses presents an increasingly difficult problem for the meat packer. Use of the expressed fluids from rumen contents for feed supplements and the residue as a fuel might provide an economically sound solution to the problem.

The addition of alfalfa ash to the corn cob ration increased the synthesis of all vitamins. This parallels the observation that alfalfa ash increases the digestibility of the organic matter of a ration (7). The bacteria of the rumen apparently need minerals to utilize the carbohydrate of the ration to the fullest extent. Whether the increased

TABLE IV

PANTOTHENIC ACID CONTENT OF RATIONS CONSUMED AND OF RUMEN CONTENTS OF SHEEP

Ration Number	Ration	Sheep Number	Present in Ration Microgms/gm. Dry Matter	Present in Rumen Contents Microgms/gm. Dry Matter	Percentage Increase
1	Prairie Hay	2 23	10.1	8.1 8.4 Ave. 8.3	 Ave.
2	Prairie Hay, Corn, Urea	5 11	8.0	14.8 13.7 14.3	85 71 78
3	Prairie Hay, Corn, Soybean Meal	6 18	9.5	17.3 17.7 17.5	82 86 84
4	Silage, Corn, Soybean Meal	7 12_2	15.2	12.6 20.1 16.4	 8
5	Alfalfa, Corn, Soybean Meal	10 29	16.5	23.4 21.8 22.6	42 32 37
6	Corn, Soybean Meal	4 14	7.4	32.2 27.9 30.1	335 277 306
7	Corn Cob Basal	8 12_1	5.2	17.5 15.2 16.4	237 192 215
8	Corn Cob Basal + Alfalfa Ash	1 13	5.2	15.4 22.0 18.7	196 323 260

vitamin content resulted from the increased microbial population associated with more complete digestion, or whether it may have been in part, responsible for creating a more favorable environment with more vigorous fermentation is unknown. It seems significant, however, that both increased digestion of the roughage and increased vitamin content were induced by the addition of a highly complex mineral mixture. Further studies to illustrate the mechanism of the condition seem warranted.

Due to the wide variation in the amounts of vitamin found in the rumen contents of sheep on the same ration, and the small number of animals used in this study, the results are not too significant.

TABLE V

AVERAGES OF AMOUNTS OF VITAMINS AND PERCENTAGE INCREASES

Ration	Amount in Ration Microgms/gm. Dry Matter	Amount in Rumen Contents Microgms/gm. Dry Matter	Percentage Increase
	Riboflavin		
1	3.3	6.2	88
2	2.2	8.6	290
3	2.4	9.5	296
4	3.2	13.7	327
5	5.2	11.7	125
6	1.5	9.9	560
7	1.8	6.4	256
8	1.8	8.2	356
	Nicotinic Acid		
1	14.5	51.4	254
2	15.2	84.1	453
3	16.4	100.4	513
4	21.1	148.6	604
5	23.4	138.8	493
6	16.9	108.4	542
7	18.6	68.2	267
8	18.6	86.9	367
	Pantothenic Acid		
1	10.1	8.2	
2	8.0	14.3	78
3	9.5	17.5	84
4	15.2	16.4	8
5	16.5	22.6	37
6	7.4	30.1	306
7	5.2	16.4	215
8	5.2	18.7	260

SUMMARY

The rumen contents of sheep receiving various rations were analyzed microbiologically for riboflavin, nicotinic acid, and pantothenic acid. Prairie hay alone promoted very little synthesis of these three vitamins in the rumen. An 88% increase of riboflavin, a 254% increase for nicotinic acid, and an apparent decrease in pantothenic acid as compared to the amounts in the ration consumed was found. The addition of energy and protein to the ration greatly increased the synthesis. Increases of 290%, 453%, and 78% above the amounts present in the ration were found for riboflavin, nicotinic acid, and pantothenic acid, respectively.

The quality of roughage fed affected the synthesis of vitamins to a slight extent, riboflavin and nicotinic acid particularly being favored by feeding silage. However, the absolute amounts of vitamins present had a tendency to be stabilized if energy and protein were present in adequate amounts. A readily available carbohydrate appeared to increase the synthesis of all vitamins. The addition of alfalfa ash to a corn cob ration increased the synthesis of riboflavin, nicotinic acid, and pantothenic acid by 28, 27, and 14%, respectively.

The quantity of these vitamins in the rumen contents appears to be too small to make economical the processing of rumen contents to recover the vitamins, if the value of the vitamins recovered is considered as the sole source of income.

II. THE INFLUENCE OF FERTILIZATION ON THE AMINO ACID COMPOSITION OF CEREAL GRASSES AND ALFALFA

II. THE INFLUENCE OF FERTILIZATION ON THE AMINO ACID COMPOSITION OF CEREAL GRASSES AND ALFALFA

INTRODUCTION

The influence of fertilization on the growth and vigor of forage plants is well known. Less information is available on the influence of such treatments upon the nutritive value of the plant as indicated by chemical composition. It has been claimed recently that changes in the amino acid composition of plants can be effected by fertilization. The determination of the effect of fertilization on the chemical composition of common forage plants with reference to nitrogen and amino acid composition would provide information useful in evaluating the influence of fertilization on forage crops.

MacVicar (23) and Reber (29) in studies conducted at this station have shown that the common cereal grasses contain large amounts of total nitrogen, that most of this nitrogen is present in the form of protein or amino acids, and that the amino acid distribution is such as to produce a protein of good quality.

The amino acid composition of a protein indicates its quality and thereby, to some degree, its nutritive value. The effects of fertilizers on the protein of a plant are comparable to the effects of different rations upon an animal protein. The amino acid composition of a given type of animal tissue was found by Lyman and Kuiken (20) to be very nearly the same whether it came from beef, pork or lamb with the exception of histidine which gave wide variations. The findings of Chibnall (8), Tristram (40), and Lugg and Weller (18, 19) suggest that, in general, there was a marked uniformity in the amino acid contents of the proteins contained in the leaves of herbage species. Reber (29) found that the general pattern of distribution of various amino acid in the total leaf protein was similar for most cereals.

The influence of mineral nutrients upon amino acid composition of cereal grasses and alfalfa has been studied by several workers. Sheldon et al. (32) report that considerable differences occurred in the amino acid content of lespedeza grown on five different types of Missouri soil. Alfalfa grown on a single soil exhibited, in general, an increase in amino acid content when treated with manganese, and boron, or a mixture of these with cobalt, copper, and zinc. Blue and co-workers (5) report variations in the concentration of amino acids present in alfalfa due to fertilization with Mn, B, P, and K. The minor elements Mn and B gave the greatest increase without altering the concentration of nitrogen. They interpreted such data to indicate that it was possible to improve the quality as well as the yield of the protein by relatively small application of inorganic nutrients. Tisdale et al. (39) using two clonal lines of alfalfa grown in a greenhouse on flint-shot quartz sand in nutrient solutions with concentrations of sulfate ion varying from 0 to 81 ppm. demonstrated considerable difference in their relative ability to synthesize methionine. Total nitrogen was higher at the lower levels of sulfur, decreasing as the concentration of sulfur was increased. The percentage of methionine and cystine in the alfalfa increased with increasing concentrations of sulfur. Sheldon, et al. (34) also found that the methionine content of the alfalfa increased progressively as sulfur was applied and that this increase seemed to occur at the expense of other amino acids. Sheldon and co-workers further (33) reported that the percentage of tryptophan varied widely with the inorganic composition of the substrate upon which alfalfa was grown and that the concentration of tryptophan decreased when Mg, B, Mn and Fe were withheld from the culture solutions, while synthesis of tryptophan was stimulated by increased Ca content. Smith and Agiza (35) reported that samples of first growth of the grasses and clovers contained more leucines and arginine and less glutamic acid than the second growth, and that nitrogenous fertilizers decreased the aspartic and glutamic acid yields and increased the leucines, phenylalanine, arginine, lysine and tryptophan in young rye grass and late clover.

In view of these reports that mineral nutrients cause variation in the amino acid contents of cereal grasses and alfalfa, while the make up of an animal protein remains essentially constant, it was deemed desirable to study the effect of nitrogen and phosphorus fertilization on cereal grasses and other pasture crops.

Alfalfa plots which had received various fertilizers (phosphorus, potassium, boron, and manure) were also available and preliminary studies were undertaken with this crop.

MATERIALS AND METHODS

Source of Materials. Three cereal grasses, rye (*Scale cereale*, L., var. Balbo), oats (*Avena sativa*, L., var. Wintok), and wheat (*Triticum aestivum*, L., var. Triumph); and alfalfa (*Medicago sativa*, L., var. Oklahoma Common) were used in this study. The rye, oat, and wheat grasses were produced on Kirkland silt loam of low fertility near Stillwater, Oklahoma, during the 1951-52 growing season on quadruplicate plots, randomized within each block, with the following fertilizer treatments: 1. None, 2. 40 pounds of N per acre as NH_4NO_3 applied as a top dressing after the first clipping (Code = N), 3. 40 pounds of P_2O_5 per acre as superphosphate applied at seeding (Code = P), 4. 40 pounds P_2O_5 applied at seeding and 40 pounds applied as a top dressing after the first clipping (Code = NP), 5. 80 pounds of N applied as a top dressing after the first clipping and 40 pounds P_2O_5 applied at seeding (Code = 2NP). The alfalfa was produced during the 1951-52 growing season on plots established at the Heavener Branch Station on soil deficient in phosphorus, potassium, and possibly in boron. These plots were fertilized in the spring of 1952 as follows: (A) None, (B) 320 pounds of superphosphate per acre (Code = P_2), (C) 480 pounds of superphosphate per acre (Code = P_3), (D) 320 pounds of super phosphate plus 120 pounds of muriate of potash per acre (Code = P_2K), (E) 320 pounds of superphosphate plus 120 pounds of muriate of potash plus 40 pounds of borax per acre (Code = P_2KB), (F) five tons of manure per acre (Code = M).

TABLE I

EFFECT OF FERTILIZATION ON YIELD* (LBS./ACRE) OF CEREAL GRASSES

Treatment	First Clipping	Second Clipping	Total Yield
	Balbo Rye		
None	1006	1310	2316
P	1984	1209	3193
N	1006	1343	2349
NP	1984	1496	3480
2NP	1984	1306	3290
	Wintok Oats		
None	199	2766	2965
P	481	2890	3371
N	199	2690	2889
NP	481	3680	4161
2NP	481	3387	3868
	Triumph Wheat		
None	329	1376	1705
P	705	1376	2081
N	329	1427	1756
NP	705	1492	2197
2NP	705	1441	2146

*Based on dry weight.

TABLE II

EFFECT OF FERTILIZATION ON LEAF STEM RATIO* AND YIELD OF ALFALFA

Treatment	First Clipping	Second Clipping	Total Yield
	Leaf Stem Ratio		
None	1.98	0.74	2592
P_2	2.01	0.95	3907
P_3	1.79	0.62	4280
P_2K	1.95	0.75	4500
P_2KB	1.88	0.74	4787
M	2.18	1.02	2600

*Based on dry weight.

Collection of Materials. When the cereal grasses had attained heights of from 4 to 10 inches each was clipped two inches from the ground, samples were taken for amino acid assay, and total yield (dry wt.) was determined (Table I). After the first clipping the plants were allowed to grow until they had attained a height of 8 to 14 inches and then sampled as the first clipping. After clipping the samples were taken immediately to the laboratory and dried in a forced-draft oven at 60° C. They were ground in a Wiley Mill and stored in glass air-tight bottles until analyzed for total nitrogen and amino acid composition.

The first alfalfa samples were clipped when the plants had attained a height of from 10 to 15 inches. The second samples were taken when the alfalfa was in the early blooming stage. After the second clipping the alfalfa was harvested and the yield of hay calculated, as shown in Table II. The clippings were brought to the laboratory and the leaves were separated from the stems. The leaf and stem samples were dried at 60° C. in a forced-draft oven, then weighed and the leaf-stem ratios, as presented in Table II, determined. The samples were then ground and stored as were the cereal grass samples until analyzed for total nitrogen and amino acid composition.

Sampling Procedure for Analyses. The samples for analysis of rye, oats, and wheat were pooled by fertilizer treatment. Five grams of each dried and ground sample were taken and combined to make a single composite. Total nitrogen and amino acid analyses were performed on the composite samples. For alfalfa the total nitrogen was run on each individual sample and then the samples were pooled according to treatment as described above and the amino acid analysis run on the composite samples.

Analytical Methods. Total nitrogen was determined by macro-Kjeldahl procedure according to the A.O.A.C. (2). Amino acids were determined by acid hydrolysis of the whole tissues. Hydrolysates were prepared by heating 2.5 gms. of dry material with 50 ml. of 3 N HCl in an autoclave for 10 hours at 15 pounds pressure. These samples were ajusted to pH of 7 with 6 N NaOH using a Beckman pH meter, made to volume of 100 ml. and filtered. The filtrates were analyzed for methionine and lysine using Leuconostoc mesenteroides P-60. Threonine was assayed using Streptococcus faecalis R. Lactobacillus arabinosus 17-5 was used for the assay of glutamic acid and isoleucine. For the assay of lysine, threonine, glutamic acid, and isoleucine essentially the media and procedure of Henderson and Snell (12) was employed. Essentially the procedure of Lyman et al. (21) for methionine using peroxide treated peptone media, with a citrate buffer in place of acetate, was employed. The currently available methods of hydrolysis of proteins such as were employed in this study result in indeterminable losses, particularly when large amounts of carbohydrate are present. The values presented should, therefore, be regarded as minimal particularly in the case of methionine which is probably considerably less than the actual amount. These values, however, permit a considerable degree of evaluation of the effect of various minerals on amino acid content. The content of the amino acids are given in the tables on the basis of percentage of total protein to conform with general usage, the data was calculated on the assumption that the mixed proteins contained 16 percent nitrogen and that all the Kjeldahl nitrogen represented protein.

RESULTS AND DISCUSSION

The amino acid composition of three cereal grasses (Balbo rye, Wintok oats, and Triumph wheat) and alfalfa (Oklahoma common) as effected by fertilization are presented in Tables III and VI. The values are reported as grams of amino acid from 100 grams of protein (assuming N = 16%).

Cereal Grasses

Methionine. The methionine content of Balbo rye, Wintok oats, and Triumph wheat gave ranges of 1.61 - 1.95, 1.73 - 1.97, and 1.80 - 1.87. Mean values were 1.76, 1.85, and 1.83, respectively. These values compare very favorably with the results of Reber and MacVicar (30), who found a value of 1.6 for the average of five cereal grasses, and with the value of 1.2 - 1.6 for spermatophytes as summarized by Lugg (17). There was no detectable effect of any fertilizer treatment on the methionine content of these cereal grasses.

Lysine. Balbo rye, Wintok oats, and Triumph wheat gave ranges of 4.2 - 4.6, 4.3 - 4.9, and 4.6 - 5.4 with means of 4.4, 4.6, and 4.9, respectively for lysine. These values compare with values of 5.0 - 6.8 for spermatophytes by Lugg (17) and 6.2 for cereal grasses by Reber and MacVicar (30). As in the case of methionine, there was no effect of fertilization.

Threonine. The ranges of threonine were 4.6 - 5.1, 4.8 - 5.8, and 4.2 - 5.4 for Balbo rye, Wintok oats, and Triumph wheat, respectively with mean values of 4.9, 5.4, and 4.9. These values compare favorably

TABLE III

EFFECT OF FERTILIZATION ON AMINO ACID COMPOSITION OF CEREAL GRASSES

Treatment	Nitrogen %	Amino acid from 100 gms. protein (N = 16%)				
		Methionine	Lysine	Threonine	Glutamic Acid	Isoleucine
		Balbo Rye, First Clipping				
None	3.84	1.73	4.5	5.1	10.4	4.9
P	3.32	1.80	4.5	4.8	9.5	5.1
		Second Clipping				
None	3.78	1.70	4.2	4.7	9.0	4.7
P	3.95	1.95	4.3	5.0	9.5	4.8
N	4.16	1.86	4.6	4.7	8.9	4.5
NP	4.45	1.68	4.2	4.6	9.3	4.6
2NP	4.58	1.61	4.2	4.6	9.2	4.6
		Wintok Oats, First Clipping				
None	3.67	1.73	4.8	5.5	10.5	5.5
P	3.95	1.75	4.9	5.5	9.5	5.4
		Second Clipping				
None	2.97	1.97	4.5	5.7	10.5	5.5
P	2.67	1.95	4.4	5.8	9.8	5.7
N	3.38	1.93	4.6	4.8	10.4	5.2
NP	3.47	1.81	4.7	5.3	10.3	5.2
2NP	3.57	1.78	4.3	5.0	9.9	5.2
		Triumph Wheat, First Clipping				
None	3.89	1.80	5.1	5.1	9.2	5.0
P	3.79	1.87	5.4	5.3	9.5	5.4
		Second Clipping				
None	3.33	1.87	4.7	5.4	10.0	5.2
P	3.49	1.86	4.9	5.2	9.9	4.9
N	3.52	1.80	4.7	4.2	9.4	4.7
NP	3.52	1.81	4.8	4.7	9.6	4.7
2NP	3.75	1.81	4.6	4.7	9.6	4.7

with the 4.8 found by Reber and MacVicar (30), but are somewhat higher than the 3.0 - 4.0 summarized by Lugg (17). There was no effect of fertilization.

Glutamic Acid. The glutamic acid content of Balbo rye, Wintok oats and Triumph wheat varied between 8.9 - 10.4, 9.8 -10.5, and 9.2 - 10.0 with mean values of 9.4, 10.1, and 9.6, respectively. These values were somewhat lower than the 11.7 of Reber and MacVicar (30), but higher than the 6.5 - 7.8 value of Lugg (17). Fertilization did not effect the glutamic acid content of these grasses.

Isoleucine. Balbo rye, Wintok oats, and Triumph wheat gave ranges of 4.5 - 5.1, 5.2 - 5.7, and 4.7 - 5.4 with mean values of 4.7, 5.4, and 4.9, respectively for isoleucine. These values compare with 5.8 found by Reber and MacVicar (30), and 7.1 - 3.6 summarized by Lugg (17). The isoleucine content was not altered by fertilization.

The total yield as shown in Table I and the protein as shown by the percent nitrogen in Table III for all three cereal grasses increase with nitrogen and phosphorus fertilization, but the distribution of the amino acids assayed for in this study remain essentially the same for all treatments.

Alfalfa

Methionine. The methionine content for alfalfa leaves and stems ranged between 1.09 - 1.22, and 0.72 - 0.93 respectively with mean values of 1.16 and 0.84. Assuming a leaf stem ratio of 1.0, the average value for the entire plant, 1.0, compares with the value of 0.91 for the whole plant by Armstrong (1). The 1.16 for leaves compares with 1.2 - 1.4 for *Leguminosae* leaves as summarized by Lugg (17).

TABLE IV

COMPARISON OF AMINO ACID COMPOSITION OF VARIOUS CEREAL GRASSES AS AFFECTED BY FERTILIZATION

Amino acid from 100 gms. protein (N = 16%)

Clipping Number	Cereal	Treatment				
		None	P	N	NP	2NP
				Methionine		
1	Rye	1.73	1.80			
2	Rye	1.70	1.95	1.86	1.68	1.61
1	Oats	1.73	1.75			
2	Oats	1.97	1.95	1.93	1.81	1.78
1	Wheat	1.80	1.87			
2	Wheat	1.87	1.87	1.80	1.81	1.81
				Lysine		
1	Rye	4.5	4.5			
2	Rye	4.2	4.3	4.6	4.2	4.2
1	Oats	4.8	4.9			
2	Oats	4.5	4.4	4.6	4.7	4.3
1	Wheat	5.1	5.4			
2	Wheat	4.7	4.9	4.7	4.8	4.6
				Threonine		
1	Rye	5.1	4.8			
2	Rye	4.7	5.0	4.7	4.6	4.6
1	Oats	5.5	5.5			
2	Oats	5.7	5.8	4.8	5.3	5.0
1	Wheat	5.1	5.3			
2	Wheat	5.4	5.2	4.2	4.7	4.7
				Glutamic Acid		
1	Rye	10.4	9.5			
2	Rye	9.0	9.5	8.9	9.3	9.2
1	Oats	10.5	9.5			
2	Oats	10.5	9.8	10.4	10.3	9.9
1	Wheat	9.2	9.5			
2	Wheat	10.0	9.9	9.4	9.6	9.6
				Isoleucine		
1	Rye	4.9	5.1			
2	Rye	4.7	4.8	4.5	4.6	4.6
1	Oats	5.5	5.4			
2	Oats	5.5	5.7	5.2	5.2	5.2
1	Wheat	5.0	5.4			
2	Wheat	5.2	4.9	4.7	4.7	4.7

Lysine. Alfalfa leaves and stems had a lysine content ranging between 4.7 - 5.4 and 4.5 - 5.2, respectively with mean values of 5.0 and 4.8. These values are more than twice as high as the 2.10 for Lucerne by Armstrong (1). The value for the leaves is somewhat lower than the 6.4 - 6.5 for Leguminosae leaves as reported by Lugg (17).

Threonine. The values for threonine ranged between 4.6 - 5.9 and 4.3 - 4.9 for alfalfa leaves and stems, respectively with mean values of 5.4 and 4.6, which are some higher than 4.0 for Leguminosae leaves reported by Lugg (17).

Glutamic Acid. The glutamic contents for alfalfa leaves and stems ranged between 8.8 - 9.7 and 7.1 - 7.9, respectively with mean values of 9.2 and 7.4. The values for the leaves are higher than the 6.4 - 6.7 for Leguminosae reported by Lugg (17).

Isoleucine. The values for isoleucine ranged between 5.0 - 5.6 and 4.1 - 4.9 for alfalfa leaves and stems respectively with mean values of 5.3 and 4.6. Lugg (17) reported a value of 3.6 for Leguminosae leaves.

The total yield as shown in Table II was greater on the plots where fertilizer was applied, but there was no effect on the amino acid composition of either the leaves or the stems. That the leaf and stem protein of alfalfa are different in amino acid composition has been demonstrated in these studies. The stem protein contained less methionine, 0.84 vs. 1.18; less threonine, 4.6 vs. 5.4; less glutamic acid, 7.4 vs. 9.2; and less isoleucine, 4.6 vs. 5.3; than the leaf protein. As the plant matured the leaf-stem ratio, as shown in Table II, decreased from 2.0 to 0.8. This would cause a shift in the relative proportion of the amino acids of the whole plant protein derived from leaf tissue. Differences in amino acid composition of an entire plant, attributed to

TABLE V

EFFECT OF FERTILIZATION ON AMINO ACID COMPOSITION OF ALFALFA

Treatment	Nitrogen %	Amino acid from 100 gms. protein (N = 16%)				
		Methionine	Lysine	Threonine	Glutamic Acid	Isoleucine
		Alfalfa Leaves, First Clipping				
None	4.68	1.20	4.8	5.9	9.3	5.3
P_2	4.85	1.18	5.1	4.6	8.8	5.1
P_3	5.08	1.14	5.0	5.2	9.1	5.2
P_2K	4.95	1.22	5.1	5.5	9.7	5.3
P_2KB	4.99	1.15	4.9	5.7	9.4	5.0
M	4.71	1.21	5.0	5.7	9.5	5.5
		Second Clipping				
None	3.95	1.16	4.7	5.6	9.1	5.3
P_2	3.86	1.16	4.8	5.7	9.0	5.6
P_3	4.03	1.10	5.1	4.9	8.8	5.2
P_2K	3.65	1.09	5.4	5.1	9.1	5.4
P_2KB	3.84	1.10	5.0	5.2	9.6	5.4
M	3.98	1.16	5.3	5.5	8.9	5.4
		Alfalfa Stems, First Clipping				
None	3.95	0.88	4.7	4.5	7.3	4.3
P_2	3.86	0.87	5.0	4.6	7.4	4.6
P_3	3.97	0.80	4.7	4.6	7.1	4.1
P_2K	3.75	0.84	4.7	4.3	7.8	4.7
P_2KB	3.73	0.91	4.7	4.8	7.3	4.5
M	3.68	0.90	5.2	4.5	7.7	4.6
		Second Clipping				
None	1.60	0.93	5.0	4.8	7.9	4.9
P_2	1.80	0.84	4.6	4.8	7.2	4.7
P_3	1.88	0.72	4.6	4.5	7.1	4.6
P_2K	1.67	0.84	4.6	4.4	7.2	4.1
P_2KB	1.67	0.86	4.9	4.9	7.9	4.9
M	1.66	0.73	4.5	4.7	7.4	4.7

TABLE VI

COMPARISON OF AMINO ACID COMPOSITION OF ALFALFA LEAVES AND STEMS AS AFFECTED BY FERTILIZATION

Amino acid from 100 gms. protein (N = 16%)

Clipping	Treatment					
	None	P_2	P_3	P_2K	P_2KB	M
			Methionine			
1 Leaves	1.20	1.18	1.14	1.22	1.15	1.21
2 Leaves	1.16	1.16	1.10	1.09	1.10	1.16
1 Stems	0.88	0.87	0.80	0.84	0.91	0.90
2 Stems	0.93	0.84	0.72	0.84	0.86	0.73
			Lysine			
1 Leaves	4.8	5.1	5.0	5.1	4.9	5.0
2 Leaves	4.7	4.8	5.1	5.4	5.0	5.3
1 Stems	4.7	5.0	4.7	4.7	4.7	5.2
2 Stems	5.0	4.6	4.6	4.6	4.9	4.5
			Threonine			
1 Leaves	5.9	4.6	5.2	5.5	5.7	5.7
2 Leaves	5.6	5.7	4.9	5.1	5.2	5.5
1 Stems	4.5	4.6	4.6	4.3	4.8	4.5
2 Stems	4.8	4.8	4.5	4.4	4.9	4.7
			Glutamic Acid			
1 Leaves	9.3	8.8	9.1	9.7	9.4	9.5
2 Leaves	9.1	9.0	8.1	9.1	9.6	8.9
1 Stems	7.3	7.4	7.1	7.8	7.3	7.7
2 Stems	7.9	7.2	7.1	7.2	7.9	7.4
			Isoleucine			
1 Leaves	5.3	5.1	5.2	5.3	5.0	5.5
2 Leaves	5.3	5.6	5.2	5.4	5.4	5.4
1 Stems	4.3	4.6	4.1	4.7	4.5	4.6
2 Stems	4.9	4.7	4.6	4.1	4.9	4.7

fertilization, might be secondary to a variation in ratio of plant parts. Care should, therefore, be exercised in the interpretation of data correlating composition and fertilizer treatment. Whether such changes in lespedeza and alfalfa were responsible for the differences reported by Sheldon, *et al.* is not known, since no yield data is presented by them. It would seem desirable, however, to reserve judgment on the effect of fertilizer treatment on the amino acid composition of the plant until careful study is made of other possible effects of fertilization on growth and development of the plant. It would seem particularly desirable to avoid attributing changes in amino acid composition to any direct effect of the fertilizer *per se* on the synthesis of a particular amino acid.

SUMMARY

Two clippings of three cereal grasses (rye, oats, and wheat) were analyzed microbiologically for the following amino acids: methionine, lysine, threonine, glutamic acid, and isoleucine to determine the influence of nitrogen and phosphorus fertilization on the composition of leaf protein. All varieties had a very similar pattern of distribution of these amino acids in the bulk proteins of the clippings, although the total protein and yield were both increased. There was no apparent alteration in the amino acid pattern due to either nitrogen or phosphorus fertilization. Average values for all varieties for both samplings (amino acid expressed as grams from 100 gms. of protein, assuming N = 16 percent) are as follows: methionine, 1.81; lysine, 4.6; threonine, 5.1; glutamic acid 9.7; and isoleucine, 5.0.

The leaves and stems of alfalfa from two clippings were analyzed separately for the same amino acids as the cereal grasses to determine the effect of phosphorus, potassium, boron, and manure fertilization on the amino acid pattern of the leaf and stem proteins. Although the total yield was increased by fertilization, the amino acid composition of the leaves and stems separately did not change appreciably. Due to differences in composition between the leaf stem protein, amino acid composition of the entire plant did shift with maturity. A change in the leaf-stem ratio from approximately 2.0 to 0.8 would cause an obvious

shift in the amino acid pattern of the plant taken as a whole. Average values for both clippings (amino acids expressed as grams amino acid from 100 grams of protein assuming N = 16%) were as follows: for leaves, methionine, 1.16; lysine, 5.0; threonine, 5.4; glutamic acid, 9.2; and isoleucine, 5.3; for stems, methionine, 0.84; lysine, 4.8; threonine, 4.6; glutamic acid, 7.4; and isoleucine, 4.6.

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II. INFLUENCE OF FERTILIZATION ON THE AMINO ACID COMPOSITION OF CEREAL GRASSES AND ALFALFA

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II. THE INFLUENCE OF FERTILIZATION ON THE AMINO ACID COMPOSITION OF CEREAL GRASSES AND ALFALFA

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