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A STUDY OF THE CYANOGENESIS IN SORGHUM VULGARE

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INTRODUCTION

It is a prevalent belief among farmers, and also among certain writers on the subject of sorghums, that when the sorghum is cut and cured it is no longer poisonous to stock. While this is a strong belief among farmers, and is stated as a fact by certain writers and investigators, yet there are other writers and investigators who have claimed that curing has no effect on the power of sorghum to poison stock. In fact, the literature on this subject is quite conflicting in its statements. For instance, Churchill (4) states that sorghum is rendered safe for feeding by curing. The editor of the Journal of the Department of Agriculture of Victoria (10) states that in curing, the sorghum is rendered harmless. On the other hand, Schroder and Dammann (7), and also Brunnich (3), claim that the sorghum is not rendered harmless in the curing process. Madison (13) makes the following statement: "Second-growth sorghums may develop prussic acid, which is poisonous to stock. The first crop, however, seldom ever develops poison, nor is this found in the dry forage." Furthermore, the well known fact is recalled in this connection that linseed cake and certain varieties of beans are known to contain prussic acid in the form of glucoside. Avery and Peters (2) were not sure whether the sorghum is rendered suitable for feeding by curing, and stated that the subject should be further investigated.

During this past summer, reports came to this Station, through the newspapers, of several cases of poisoning caused by sorghum which had been cut for some time. This information, the fact that several inquiries were made by farmers as to whether or not it would be safe to feed sorghum which had been cut during dry weather, and the lack of definite information in the literature, caused me to take up the present investigation.

There are several questions that should be investigated. The first and probably the most important, is to determine whether or not the glucoside is decomposed and the prussic acid liberated when the sorghum is cured; 2, to determine whether or not the enzyme is rendered inactive in the process of curing, as claimed by Avery and Peters (2); 3, to determine the effect of the presence of substances such as glucose and maltose on the liberation of the prussic acid from the gluco-

side, and 4, to determine whether or not the prussic acid may be present in more than one form, as has been claimed by Willamann (9). While these are the main points studied, there are several others possibly of minor importance that were studied.

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EXPERIMENTAL

Four different samples of sorghum were used. One was a sample obtained from Mr. Ed Singleton of Chickasha, and was a part of a lot of sorghum which had been cut when it was about 2½ feet high, and at a time when there was an extreme drouth in the southwestern part of the State. This was a part of some sorghum which had been fed to twelve head of cattle, ten of which had died within one hour. This will be called sample No. 1. Sample No. 2 was cut at the same stage of growth by Mr. P. A. Gould of Stillwater, but had not been subjected to as extreme drouth as Sample No. 1, since it was cut at the beginning of the dry weather. Sample No. 3 was a second-growth sorghum which had grown after heavy rains had fallen, and there had been plenty of moisture in the ground all during its growth. This sample was cut fresh each time as it was needed, and was about knee-high at the time of cutting. Sample No. 4 was a volunteer sorghum cut from the Experiment Station farm. This sample was in the dough stage when cut, but it had been subjected to the dry weather of the summer and had grown quite vigorously after the rains had fallen.

The method of determining the prussic acid was a modification of that used by Viehoever and Johns (8), and by Knight (5). In the case of the dry samples, Nos. 1 and 2, the sorghum was cut into fine pieces and then run through a feed mill. Samples Nos. 3 and 4 were cut a little at a time, this part being thoroughly wet and bruised in a large iron mortar. The bruised portions were placed in water in the digestion flask. At first each of these samples were kept in digestion flasks in a water bath at 40° C. for two hours, the apparatus being so arranged that any prussic acid which passed off would be collected in sodium hydroxide. After this period of digestion the water bath was removed and 100 cc. were distilled as rapidly as possible, the prussic acid being collected in sodium hydroxide. It was found by two or three trials that all of the prussic acid was driven over by distilling 100 cc. At first the distillate was evaporated in vacuum, as directed by Viehoever and Johns (8), but since this required such a long time it was decided to carry on the evaporation by placing the distillate in a flat form evaporation dish on a water bath which was heated by an electric hot plate. A current of air from an electric fan on low speed was directed across the evaporating dish. It was found that under these conditions the solution was usually at about 60° C., and in no case did the temperature go above 70° C. With such an arrangement the evaporation could be made easily within two hours. After the distillate was evaporated almost to dryness, freshly prepared ferrous

sulphate was added and acidified with 30% nitric acid, as directed by Viehoever and Johns (8). Instead of filtering the prussian blue precipitate into a Gooch crucible, as was done by Knight (5), it was filtered in the ordinary way and washed thoroughly with dilute nitric acid and then with water. The precipitate and filter paper were then placed in a flat form platinum dish and heated slowly to dryness in an electric muffle furnace, and then heated strongly to burn the precipitate and oxidize the iron. The dish containing the residue, consisting of the ash of the filter paper and the ferric acid, was weighed. From the weight of the ferric oxide the amount of prussic acid was calculated, and from this the percent of prussic acid in the dry sorghum. The percent of moisture in the different samples of sorghum was found by drying at 105° C.

No effort was made to determine whether or not this method would give accurate results, but it was thought that the results would be as accurate as those obtained in using Knight's method (5) and the colometric methods of Viehoever and Johns (8), and of Francis and Connell (11), could not be used, since a colorimeter was not available. Moreover, it was thought that this method would give results sufficiently accurate for comparative purposes.

In order to determine whether or not a part of the prussic acid was lost in the drying, Sample No. 3 was cut and digested, as described above, and then some of it was allowed to dry in the laboratory for two and one-half days, and was then placed on top of a Freas oven over night. The temperature on top of this oven was 33° C. A part of this sample was used for the determination of prussic and another for the determination of the water still present.

In order to determine the effect of the rate of drying on the loss of prussic acid, if any, another part of Sample No. 3 was dried at 50° C. within twenty-four hours. The results obtained here are given in the table below under Experiments Nos. 1, 2 and 3.

To determine the effect of the presence of glucose and maltose on the liberation of the prussic acid, portions of Sample No. 2 were digested in a solution containing 1% of dextrose and 1% of maltose. The results of two trials here are given in the table under Experiment No. 5.

To determine whether or not a part of the prussic acid existed in the form of non-glucosidic, as has been claimed by Willaman (9), portions of Samples Nos. 2 and 3 were digested and 200 cc. distilled off and then 50 cc. of 10% sulphuric acid was added to the digestion mixture, which had a volume of about 800 cc., and another 100 cc. was distilled. This last distillate was evaporated and tests made for prussic acid with negative results, as indicated in the table under Experiment No. 6.

It has been pointed out by Auld (1) that with most feedstuffs digestive conditions would be unfavorable for the action of the enzyme on the glucoside, but he points out very correctly that a slight acidity

is the best condition for the action of the enzyme, and that this acid condition might be found in the paunch of ruminants when certain feedstuffs are used. This being true, it was important to know the acidity of the juice of the sorghum. The juice was pressed from portions of Samples Nos. 3 and 4 and portions of this juice were diluted very much and titrated with sodium hydroxide, using phenolphthlein. Our results here are given in the table under Experiment No. 7. The acids present in the Samples Nos. 1 and 2 were not determined, but it is quite probable that all the acids present were non-volatile and remained in the dry sorghum. Several other determinations of lesser importance were made, the results of which will be found in the table:

TABLE

Experiments showing the cyanogenesis in dry and fresh sorghum under various conditions:

No. of Experiment	Description of Experiment	Percent of Prussic Acid Found
No. 1-a	Sample No. 3, digested in water at 40° C. for 1 hour	.0221
No. 1-b	Sample No. 3, same as 1-a	.0228
No. 2-a	Sample No. 3, dried for two and one-half days in the laboratory, then dried for 16 hours at 33° C	.0050
No. 2-b	Sample No. 3, same as 2-a	.0069
No. 3-a	Sacple No. 3, dried at 50° C. for 24 hours, sample thoroughly dried	.0109
No. 3-b	Sample No. 3, same as 3-a	.0070
No. 4-a	Sample No. 2, plus emulsion digested at 40° for 2 hours	.0222
No. 4-b	Sample No. 2, same as 4-a, except no emulsion present	.0130
No. 4-c	Sample No. 1, same as 4-a	.0514
No. 4-d	Sample No. 1, same as 4-b	.0450
No. 5-a	Sample No. 2 in a solution of 1% dextrose and 1% maltose	.0038
No. 5-b	Sample No. 2, same as 5-a	.0059
No. 6-a	Sample No. 3, digested for 1 hour at 40° C., distilled off 200 cc., then added 10 cc. of 10% sulphuric acid and distilled another 100 cc., test for prussic acid in last distillate	None
No. 6-b	Sample No. 2, same treatment as 6-a	None
No. 7-a	Titrated juice from Sample No. 3, normally found to be .013 normal	
No. 7-b	Normality equals .0507	
No. 8	Sample No. 4, digested for 2 hours at 40° C	.0087
No. 9-a	Sample No. 2, digested for 2 hours in 5% tartaric acid	.0119
No. 9-b	Sample No. 1, treatment same as 9-a	.0018
No. 9-c	Sample No. 3, treatment same as 9-a, except sample was ground under 5% tartaric acid	None
No. 10-a	Sample No. 3, kept at 40° C. for 15 minutes	.0281
No. 10-b	Sample No. 2, same treatment as 10-a	.0177
No. 11-a	Sample No. 2, treated with water at 80° C. and kept at this temperature for 1 hour	.0049
No. 11-b	Sample No. 2, treated with water at 90° C. and kept at this temperature for 1 hour	.0040
No. 12-a	Sample No. 2, kept in air bath at 70° C. for 1 hour	0124
No. 12-b	Sample No. 2, kept in air bath at 115° C. for 1 hour and 30 minutes	.0087
No. 13-a	Sample No. 1, kept for 1 hour in N-100 sodium hydroxide made acid and distilled	.0220
No. 13-b	Sample No. 3, kept in solution of sodium hydroxide of N-100 for 1 hour	.0051
No. 13-c	Sample No. 3, treatment same as 13-b except the sodium hydroxide was N-50, and the solution was made slightly acid with tartaric acid, and kept for 1 hour at 40° C	.0136

DISCUSSION OF RESULTS

Any discussion of the experimental results will necessarily be of the nature of a summary. A comparison of the percent of prussic acid found in the Experiments 1-a and 1-b with those in 2-a and 2-b will show that approximately three-fourths of the prussic acid is set free in the process of drying. This goes to confirm the common belief that sorghum is safe for feeding after it is dried. At the same time the results show that not all of the prussic acid disappears. A comparison of Experiments 2-a and 2-b with 3-a and 3-b shows that the rapidity with which the sorghum is dried determines the percent of the prussic acid that is retained by it. This point is of considerable importance in this State on account of the fact that farmers quite frequently cut their sorghum during drouths after it has been partially dried while yet standing, and after it is cut, being already partly dry, it dries very quickly. Under such conditions a large percent of the prussic acid would be retained in the fodder. Sample No. 1 was cut under such conditions.

A glance at Experiments 4-a, b, c and d will show that the enzyme which is present in the sorghum is still active, and that the addition of emulsin does not cause the prussic acid to be liberated in greater quantity.

A comparison of the amount of prussic acid found in Experiments 5-a and b with Experiment 4 shows that the addition of such a small quantity as 1% of dextrose and 1% of maltose seems to hold back or prevent the liberation of about three-fourths of the prussic acid. This is an extremely important result from the practical standpoint, and dextrose and maltose were selected because of the fact that they are formed by the action of the ptyalin on the starches in the paunch. This retention of the prussic acid in the presence of these sugars may be assumed to be due either to a reaction between the sugars (aldehydes) and prussic, or to a lessening of the activity of the enzyme by the sugars. This would lead to the suggestion that in case there is any doubt about the poisonous nature of the sorghum, one should feed some concentrate before feeding the sorghum. In this way a considerable quantity of dextrose and maltose would be produced by the salivary digestion and would tend to prevent liberation of the prussic acid of the sorghum which is fed afterward. When this experiment was done I had not read Avery and Peters work (2) in which they showed that it was possible to give very large doses of prussic acid without any harmful effects, provided at the same time a somewhat proportionate amount of dextrose was given.

It has been claimed by Willaman, as has already been stated, that the prussic acid exists in the sorghum in two forms, glucosidic and non-glucosidic. It seems natural to suppose that the non-glucosidic acid would not be liberated under the conditions that existed in our work, that is, the digestion was carried on in a very faintly acid solution, the acidity being due to the acids present in the sorghum. If we

make this assumption, our results in 6-a and 6-b seem to show that no non-glucosidic prussic acid exists in the sorghums. Of course it is possible that the non-glucosidic acid was distilled over in the first 100 cc., but this would not be in harmony with Willaman's supposition that it is the prussic acid that is obtained in 5% tartaric distillation that causes the poisonous effect and which is a non-glucosidic acid. Furthermore, the fact that no prussic acid was found in the distillate from Sample No. 3 when it was ground under 5% tartaric acid and distilled from the acid solution shows non-glucosidic acid is not present.

The results in Experiments 9-a and 9-b show that when a dry sorghum is digested with 5% tartaric acid a considerable percent of the prussic acid is not liberated, and when this is taken in connection with Experiment 9-c, one may conclude that the water was absorbed by the dry substance more rapidly than the acid, and that some prussic acid was set free before the acid came in contact with the glucoside.

It is seen from the acid concentrations as found in Experiments 7-a and 7-b that the contents of the paunch would be faintly acid in reaction when the green or the dry sorghum is eaten. It might be argued that the acidity would be neutralized by the alkalinity of the saliva, but when the acidity as found here is compared with the alkalinity of the saliva it is seen that, when the alkalinity of the saliva is taken into account, and assuming a normal saliva flow, the contents of the paunch would still be slightly acid, a condition most favorable for enzyme action. This acid condition would exist until rumination takes place when the acid would be neutralized.

A comparison of the results of Experiments 10-a and 10-b with that of Experiment No. 1 shows that the prussic acid is all liberated within the first fifteen minutes of the digestion.

Willaman and West (12) and other investigators have shown that prussic acid gradually disappears from sorghum during its growth so that but little is present in the mature plant. It was thought that this might not be true if large amounts of the acid had been formed in the sorghum, due to dry weather, at some stage of growth. Sample No. 4 had been stunted by dry weather, but it is seen from Experiment No. 8 that nearly all of the prussic acid had disappeared. The percent of prussic acid found in this sample should be compared with that of Sample No. 1, which was doubtless greater still before the sample was dried.

No discussion is needed of the Experiments Nos. 11 to 13-b, inclusive. The reason for doing Experiment 13-c was that it was thought that possibly, as shown in our work, the enzyme is rendered practically inactive by dilute alkaline solution, and it might be that the prussic acid would not be liberated on this acount in the paunch, but when it later entered the true stomach, where the solution would become slightly acid, the prussic acid would be set free. The result under Experiment No. 13-c seems to show that this is true. Digestion first

with N-100 sodium hydroxide, as shown in Experiment 13-b, prevents the liberation of the prussic acid. Certainly then, digestion with N-50 sodium hydroxide would prevent the liberation of this acid, and yet it is seen by acidifying this solution and allowing further digestion that more than one-half of the prussic acid was given off.

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