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A Hemorrhagic Factor in Moldy Lespedeza Hay

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ABSTRACT

Cases of hemorrhage in Missouri livestock have been reported which were suggestive of sweet clover disease even though the diets did not contain sweet clover. A case of losses from hemorrhage following dehorning of cattle receiving a ration of mill feed, sorgo silage, and moldy lespedeza hay was investigated. The hemorrhagic animals were found to have a long prothrombin time but the condition could not be traced to a genetic origin. The moldy lespedeza hay was the only ingredient of the diet that would produce a similar condition when fed to rabbits. Although the rabbits lost considerable weight on the moldy hay diet the hemorrhagic condition and low prothrombin was not due to starvation because rabbits receiving a reduced amount of good alfalfa hay maintained normal hemostasis and an adequate amount of prothrombin. The rabbits refused to eat a sufficient quantity of the moldy hay to produce a severe bleeding condition unless it was mixed with "cerelose" (corn sugar). A bleeding condition severe enough to cause death from a minor injury was produced in some of the rabbits after long periods on the sweetened moldy lespedeza hay. However, lespedeza hay that was properly cured did not produce the hemorrhagic condition.

A Hemorrhagic Factor in Moldy Lespedeza[1] Hay

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Losses occur in the livestock industry due to excessive hemorrhage which may occur under accepted management practices such as castration, dehorning, docking, etc., as well as from accidental injuries. If the injury is too severe, even normal animals will bleed to death. However, if animals have sweet clover disease, or any other derangement that interferes with hemostasis, a fatal loss of blood may result from apparently inconsequential tissue damage.

The hemorrhagic condition which occurs in sweet clover disease has received considerable attention (Link, 1943). Cattle and some other animals when fed spoiled sweet clover hay develop a hemorrhagic disease characterized by a prolonged clotting time and low prothrombin content of the blood. This condition was first noticed by Schofield (1922) in the province of Ontario and later by Roderick (1929) in the state of North Dakota. Schofield (1924) demonstrated that the blood from animals that had consumed moldy sweet clover hay showed delayed clotting. Later Roderick (1931) added further information concerning the pathology of the disease, including the fact that the prothrombin is markedly reduced and this is responsible for the delayed coagulation of the blood. Quick (1937) aided in the solution of this problem by developing a suitable assay method for prothrombin. Stahmann, Huebner and Link (1941) isolated, identified, and synthesized the active toxic principle involved, 3, 3-methylenebis-(4-hydroxy coumarin), more commonly known as dicumarol.

EXPERIMENTAL

Several cases (6 recorded) suggestive of sweet clover disease were reported in Missouri in which the diets did not contain sweet clover. One such case occurred in a herd of cattle near the University and investigations were conducted on this group of animals and their feed. In the first reported local case excessive hemorrhage had occurred following dehorning. Although veterinary services were secured, one animal died from excessive hemorrhage and another was sent to the slaughter house in a weakened condition due to the loss of

[1]Lespedeza stipulacea sometimes referred to as Korean Lespedeza.

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blood. A third animal was lost at a later date due to excessive hemorrhage at time of parturition.

Blood samples were drawn from three of the remaining animals in the herd and tested for coagulability. Animals from different lines of breeding were selected to ascertain the possibility of a genetic factor. Samples were also secured from three animals in the University herd to be used as controls. Prothrombin time and prothrombin activity were determined by the one-stage method described by Smith et al. (1939). The whole blood coagulation time was determined by the Lee-White (1913) modification of Howell's method, except that the coagulation time was determined at about 0° C. The bleeding time into saline was determined as described by Copley and Lalich (1942). A spring release blood lancet was used to make a prick wound in the tip of the ear. The ear tip was then immersed in isotonic saline at 37° C. and the bleeding time measured with a stop watch from the moment the wound was inflicted until the flow of blood stopped. The cell volume was determined by the Wintrobe (1929) method in a hematocrit tube. The fibrin precipitation time was determined by the method of Muhrer et al. (1942). This is essentially a diluted plasma coagulation time. The quantity of fibrinogen was determined by converting it into fibrin, stirring out with a glass rod, washing the fibrin free of plasma and analyzing for nitrogen content by a micro-Kjeldahl method. The amount of fibrin protein was calculated by the usual methods from the amount of nitrogen found.

The results of the tests are summarized in Table 1.

TABLE 1. -- DIAGNOSTIC OBSERVATIONS ON CATTLE

Tests	Hemorrhagic Cattle (3)	University Cattle (3)
Prothrombin Time	7 minutes	2 1/2 minutes
Prothrombin Activity (in % of normal)	35.7%	100%
Whole Blood Coagulation Time at near 0° C.	138 minutes	79 minutes
Saline Bleeding Time	600 + seconds	310 seconds
Cell Volume	33%	45%
Fibrin Precipitation Time	53 minutes	31 minutes
Amount of Fibrinogen Present	.637%	.666%

As will be noted, the prothrombin time, whole blood coagulation time, and saline bleeding time of the animals in the affected herd were increased.

This was regarded as being due to the reduced amount of prothrombin in the blood. The amount of fibrinogen present is within the normal range which eliminates liver damage as the cause since both the fibrinogen and prothrombin will be reduced when liver damage is present. The reduced cell volume in the affected cattle was assumed to be due to previous hemorrhage.

The University cattle used as controls were tested under conditions similar to those of the affected animals. The whole blood coagulation times were considerably lengthened in both groups. These increased coagulation times were due to low temperatures during the collection and testing of the samples. The relative times are more indicative of the true condition than the relationship between them and the coagulation time of samples from animals under more ideal temperature conditions. No great differences were noted between the animals of different lines of breeding, thus eliminating the possibility of a genetic factor.

The elimination of the genetic factor led us to suspect that some constituent of the feed was the cause of the hemorrhages. An examination of the ration revealed that the cattle were receiving sorgo silage, moldy mill feeds, and moldy lespedeza hay. The moldy hay was reportedly free of sweet clover. Samples of each of these were brought to the laboratory for preliminary tests. Rabbits were selected for the test animals and seven rations were prepared for the first feeding trial. The seven rations were: 1. Moldy linseed oil meal. 2. Moldy mixed dairy feed. 3. Moldy sorgo silage. 4. Moldy lespedeza hay. 5. A mixture of the four preceding feeds. 6. Basal feed. 7. Basal plus .015% dicumarol*. The basal ration contained the following constituents (all of good quality) : alfalfa meal 40 parts, bran 20 parts, soybean oil meal 15 parts, yeast 5 parts, wheat germ 5 parts, and mineral mixture 3 parts.

Preliminary to the feeding trial the prothrombin and saline bleeding times were found to be within the normal range for all rabbits to be used. These tests were conducted after the animals had been on the basal ration for a four-day preliminary feeding period. The animals were then given the diets mentioned above for a period of 15 days. Tests for bleeding tendency were conducted periodically during this time. The animals were then returned to the basal diet for 13 days, and again tested. All animals remained normal during the feeding trials except those receiving the moldy lespedeza hay and those receiving the dicumarol. Therefore the results presented in Table 2 show only a comparison of the basal ration, moldy lespedeza hay, and the basal ration plus dicumarol. The results on the other four diets were essentially the same as on the basal diet and are not included.

It will be noted that there was some decrease in prothrombin activity in rabbit Number 2 on the moldy lespedeza but not the great change noted in rabbit Number 7 getting the dicumarol. Both rabbit Number 2 and Number 7 had normal prothrombin and bleeding times at the start of the feeding trial.

*The dicumarol was kindly furnished by The Lilly Research Laboratories.

TABLE 2. -- PRELIMINARY TESTS OF FEED

Rabbit no.	Diet	Days on diet	Prothrombin		Saline bleeding time (sec.)
			Time (sec.)	% normal **	
6	Basal	32	10	100	104
2	Moldy lespedeza	15	15	66	134
7*	Basal+ .015% dicumarol	8	52	19	380

* Bled to death after being on the diet 8 days
** Prothrombin activity as described by Smith et. al. 1939.

Number 7 bled to death but Number 2 returned to normal after the moldy lespedeza ration was replaced with the basal ration. While on the moldy lespedeza hay rabbit Number 2 consumed only a fraction of the amount eaten by rabbit Number 6 on the basal diet. This was thought to be due to the unpalatability of the spoiled feed. It could not be determined whether the blood changes were due to the nutritional deficiency or to the presence of a toxic substance. Since there was such a small consumption of the moldy hay the small changes noticed in the prothrombin activity were considered significant enough to justify further investigation.

In the next feeding trial 20 rabbits were used. Six were given various amounts of a fair grade of non-moldy lespedeza hay, two the basal ration with the moldy lespedeza replacing the alfalfa and two the basal ration with red clover replacing the alfalfa. None of the animals receiving either non-moldy lespedeza, red clover or small amounts of moldy lespedeza hay showed any prolonged prothrombin times. Therefore these data are not presented in detail. The remaining ten rabbits were used in the five groups of two each presented in Table 3. Group 1 received the basal ration and Group 2 moldy lespedeza hay. In order to increase food consumption "Karo Syrup" was added to the moldy lespedeza hay for Group 3. Group 4 was given alfalfa hay only and the amount of feed restricted to the amount consumed by Group 2. This was done to determine whether or not the prothrombin time would be affected by a low food intake. Group 5 received the basal ration plus dicumarol. In order to detect smaller changes in prothrombin activity the method of Campbell et al. (1941) was used in the prothrombin determination. This method is a modification of Quick's one-stage method using diluted plasma. This dilution accounts for the longer times reported in subsequent tables starting with Table 3.

A feeding period of 19 days was used for most of the animals. The rabbits on the lespedeza hay alone consumed only a small portion compared to that eaten by the basal rabbits. One of the lespedeza fed rabbits died, apparently from starvation, in 12 days. The feeding of the dicumarol was discon-

TABLE 3. -- THE EFFECT OF KIND AND AMOUNT OF FEED UPON PROTHROMBIN TIME IN RABBITS

Group	Diet	Days on diet	Average Prothrombin Time (sec.)
1	Basal	19	26
2	Lespedeza	12*	31
3	Lespedeza plus Karo Syrup	19**	41
4	(Paired with group 2) Alfalfa	19**	28
5	Basal plus Dicumarol	5	259

*Consumed only a fraction of the amount eaten by the rabbits on basal. One died from starvation near the end of the feeding period. Blood sample impossible to secure on the remaining rabbit after the 12th day.

**Consumed only about one-third amount eaten by the rabbits on basal.

tinued after five days to prevent the unnecessary sacrificing of the animals due to fatal hemorrhages. The consumption of the lespedeza hay was considerably increased when "Karo Syrup" was added to it. In the latter group the prothrombin time was increased also and tended to indicate the presence of a toxic substance. Rabbits receiving a reduced amount of alfalfa hay maintained approximately a normal prothrombin time, thus indicating that reduced feed consumption was not a factor. The data presented in Table 3 indicate that the consumption of dicumarol produced a much greater reduction in prothrombin activity than was produced by the consumption of moldy lespedeza. However, it appeared that the greater the amount of moldy lespedeza hay consumed the greater the reduction of prothrombin activity.

In an attempt to further increase the intake of the moldy lespedeza hay, a third group of animals was placed on a feeding trial. Of the eight animals used in this trial, six were given the moldy lespedeza hay plus "Cerelose" (corn sugar) and two were maintained on the basal ration as controls. The feeding of the basal plus dicumarol was discontinued at this time and results obtained in previous trials used for comparison. The effects of consuming larger amounts of moldy lespedeza hay are shown in Table 4 and are more evident when these data are compared with those shown in Table 3.

The moldy lespedeza hay and cerelose were fed for a period of nineteen days. During this period two of the animals receiving the lespedeza hay and cerelose bled to death. One of these bled to death two days before the end of the lespedeza feeding period. Autopsy revealed intestinal hemorrhage and also hemorrage from the nostrils. The other animal died ½ hour after securing a blood sample from the ear on the nineteenth day of the feeding period. This animal showed hemorrhage in the thoracic cavity, lungs and intestinal tract.

TABLE 4. -- THE EFFECT OF MOLDY LESPEDEZA HAY ON PROTHROMBIN TIME

No. of rabbits	Ration	No. of days in feeding period (37 days total)	Prothrombin time (sec.) At end of each period
2	Basal	4	25
	Basal	7	36
	Basal	12	33
	Basal	14	28
6	Basal	4	29
	Lespedeza + Cerelose	7	40
	Lespedeza + Cerelose	12	360*
	Basal	14	26**

*Five rabbits only. One rabbit died on the 10th day of this period from hemorrhage.

**Four rabbits only. Another rabbit died from hemorrhage shortly after securing the sample at the end of the lespedeza feeding period.

Since two animals had bled to death and all the remaining animals on the moldy lespedeza and cerelose had a long prothrombin time, it was decided to change the ration back to the basal. All of the prothrombin times returned to normal within 14 days after discontinuing the feeding of moldy lespedeza hay and cerelose.

The consumption of moldy lespedeza hay by the experimental animals increased with the addition of cerelose until it was 50 per cent or more of the consumption of the basal ration by the conrol animals. The great increase in the prothrombin time which accompanied the increased consumption of the hay gave a good indication that a toxic substance, present in the moldy lespedeza hay, was the cause of the hemorrhagic condition.

The possibility of cerelose being a factor in the increased prothrombin time was considered, and another feeding trial was set up to determine whether or not cerelose was a factor. Ten rabbits were used, two receiving the basal diet, four receiving moldy lespedeza hay plus cerelose and four receiving alfalfa hay plus cerelose. The results of this feeding trial are shown in Table 5.

The data presented in Table 5 show that the prothrombin and saline bleeding times of the animals receiving the alfalfa hay and cerelose compare closely with those of the animals on the basal ration. The normal times maintained in these animals, and the marked increase in the animals receiving the lespedeza hay and cerelose eliminated the possibility of the cerelose being a factor and further indicate the presence of a toxic material in moldy lespedeza hay.

TABLE 5. -- CHANGES IN THE SALINE BLEEDING TIME AND PROTHROMBIN TIME OF RABBITS FED MOLDY LESPEDEZA HAY

No. of rabbits	Diet	Before exp. feeding		12 days on diet	19 days on diet		Return to basal for 14 days	
		S. T.	P. T.	P. T.	S. T.	P. T.	S. T.	P. T.
2	Basal	62	26	28	120	26	105	24
4	Alfalfa & Cerelose	65	25	33	112	31	82	27
4	Lespedeza & Cerelose	57	28	30	600+	272	74*	26

*Two of the animals died from hemorrhage following testing on the 19th day.
S. T. - saline bleeding time (sec.) at end of period. No determination on 12th day.
P. T. - prothrombin time (sec.) at end of period.

DISCUSSION

The occurrence of a prolonged prothrombin time and normal amounts of fibrinogen led to the belief that the cattle were receiving a toxic material that might be similar to dicumarol in physiological action. Tests on the feed indicated this toxic material to be in the moldy lespedeza. However, the results obtained in the early trials did not definitely prove that the lespedeza was at fault. At least two factors might have been responsible for the inconclusive nature of the evidence. First, the animals failed to consume sufficient amounts of the hay; and secondly, they were not allowed to remain on the diet for a time sufficient to produce a severe hemorrhagic condition. These were overcome by the addition of cerelose to the hay, which increased the consumption, and by allowing the animals to remain on the diet 19 days rather than the shorter period.

To our knowledge, saline bleeding times have not been reported in animals having a severe prothrombin deficiency due to sweet clover disease. However, in our tests, when increased prothrombin times were found they were accompanied by a proportionally prolonged saline bleeding time. This might indicate that the saline bleeding test measures a prolonged coagulation time in addition to a capillary defect or that there is a capillary defect in addition to the hypoprothrombemia.

In some experimental trials not included in the tables, good quality, bright lespedeza hay was fed to the experimental animals and no prolongation of the prothrombin time was noticed. This would indicate that the toxic substance is not found in lespedeza hay that has been properly cured and stored.

SUMMARY

1. A hemorrhagic condition in cattle of neither genetic nor sweet clover origin was observed.
2. A decreased prothrombin activity was found to be the cause of the hemorrhagic condition which in some cases was severe enough to cause death following dehorning.
3. Moldy lespedeza hay was found to be the constituent of the diet which would reproduce the condition when fed to rabbits. No abnormalities were produced when a good quality lespedeza hay was fed.
4. Rabbits were induced to consume a quantity of moldy lespedeza hay sufficient to produce the hemorrhagic condition by mixing cerelose with the moldy hay.
5. Increased consumption, and consequently a longer feeding period, than has been reported in sweet clover disease, is required to produce this condition.
6. Development of the toxic principle which produces the hemorrhagic condition is prevented by properly curing and storing lespedeza hay.

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