EXPERIMENTAL AMMONIA TOXICOSIS IN HOLSTEIN-FRIESIAN STEERS

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ABSTRACT

EXPERIMENTAL AMMONIA TOXICOSIS

IN HOLSTEIN-FRIESIAN STEERS

by Donald A. Henshaw

Toxicosis due to the oral ingestion of urea was determined in approximately 375 kg. Holstein-Friesian steers. Toxicosis from selected ammonium compounds injected intravenously was also studied. Signs associated with overconsumption of urea were atony of the rumen, polyuria, muscle tremors (ceased temporarily following urination), ataxia, tachycardia, extension of the legs, increased respiration, apparent blindness, paralysis, chronic convulsions, and death. The central nervous signs were more pronounced than with cyanide or nitrite poisoning. Diagnosis and clinical signs were associated with increased levels of blood plasma ammonia nitrogen.

Intravenous injections of ammonium carbonate, ammonium chloride, and ammonium oxalate caused clinical signs similar to those of urea toxicosis. Rumen atony occurred within 5 to 10 minutes after injection of ammonium compounds. When blood ammonia nitrogen levels increased to 0.6 mg./100 ml. external clinical signs were evident and death occurred when levels were between 1.4 and 3.2 mg./100 ml. On necropsy, no characteristic gross or microscopic lesions were observed in a steer that died from intravenous injection of ammonium compounds. Marginal perivascular and perineuronal edema in all sections of the central nervous system, vacuolation of the hepatic cells of the kidney, diffuse

hemorrhages on the mucosal surface of the abomasum and ecchymotic hemorrhages in the endocardium and myocardium were present on necropsy of the steer that died from urea toxicosis. Blood urea nitrogen levels were an unreliable indication of urea toxicosis.

This research indicated that the ammonium ion caused the toxic syndrome known as urea toxicosis.

The testing procedures for blood plasma ammonia nitrogen levels used in this research could be run in any laboratory and would be a diagnostic aid in suspected cases of urea toxicosis.

Additional determinations included blood pH and serum electrolyte levels. The results indicated that ammonium compounds injected intravenously and urea administered orally had minimal effects on blood pH or serum electrolytes.

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IN HOLSTEIN-FRIESIAN STEERS

By

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MATERIALS AND METHODS

During the course of the experiments, which will be outlined later, the following determinations were made: blood plasma ammonia nitrogen, blood urea nitrogen, blood pH and serum electrolytes (magnesium, phosphorus, calcium, chloride, sodium and potassium). Listed below are the procedures used.

Blood Plasma Ammonia Nitrogen

Whole blood was drawn from the jugular vein into a 7 ml. vacutainer tube* using sodium heparin as the anticoagulant. The sample was immediately placed in an ice bath, taken to the laboratory and centrifuged at 10 C. for 10 minutes at 2000 rpm in a model PR2 International Portable Refrigerated Centrifuge.** One milliliter of plasma was pipetted from the sample and placed in a 15 ml. centrifuge tube. The plasma sample, a known nitrogen standard, and a reagent blank were analyzed for plasma ammonia nitrogen by using the Hyland Blood ammonia kit† as described by Miller and Rice (1963). In step 7, there was a modification‡ of the Hyland test performance in that 2 ml. of 0.02 N NaOH was used. Optical

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*Becton-Dickinson and Company, Columbia, Nebraska, and Rutherford, N.J.

**International Equipment Company, Needham Heights, N.Y.

†Hyland Division, Travenol Labs, Los Angeles, California.

‡Suggested by Dr. Robert Foy, Clinical Pathologist, Edward W. Sparrow Hospital, Lansing, Michigan.

densities were determined with the model 6A Coleman Junior Spectrophotometer* at 630 mμ. The formula for plasma ammonia nitrogen determination was:

$$\frac{\text{Optical Density of Sample}}{\text{Optical Density of Standard}} \times 150 = \text{Ammonia nitrogen concentration of plasma (μg./100 ml.)}$$

The same spectrophotometer was used in subsequent experiments unless otherwise specified.

Prior to the studies on ammonia toxicosis, normal plasma ammonia nitrogen levels were determined by using 18 cattle (Table 1).

To establish if a delay in reading intervals would affect the initial plasma ammonia nitrogen levels, plasma samples from 5 Jersey cattle were read at 27 minutes, 3 Jersey cattle at 60 minutes and 18 hours, and 4 Jersey cattle at 10, 30 and 40 minute intervals.

To compare the levels of plasma ammonia nitrogen in cattle fed brome grass and orchard grass pasture versus those fed in drylot, 9 Jersey cattle were initially sampled. Five were turned out on pasture and 4 were maintained in the drylot. In 3 days all cattle were rebled and the comparisons recorded.

Blood Urea Nitrogen

Blood samples for blood urea nitrogen were collected aseptically from the jugular vein with a 5 ml. plastic syringe and transferred to a 5 ml. glass vial containing dipotassium ethylenediaminetetraacetate (EDTA) as the anticoagulant. All samples were refrigerated overnight at 4 C. Determinations were made by using the diacetyl monoxine method as described by Skeggs (1957) and Marsh *et al.* (1957). This method

*Coleman Instruments, Incorporated, Maywood, Illinois.

Table 1. Determination of average blood plasma ammonia nitrogen level in cattle

Animal No.	Breed	Age (yrs.)	Sex	Plasma Ammonia Nitrogen (µg./100 ml.)
Experimental cattle				
1	Holstein-Friesian	1	Steer	126.00
2	Holstein-Friesian	1	Steer	148.20
Other cattle				
3	Jersey	6	Cow	20.10
4	Jersey	6	Cow	43.80
5	Jersey	4	Cow	63.30
6	Jersey	3	Cow	132.60
7	Jersey	3	Cow	33.00
8	Jersey	1	Heifer	52.95
9	Jersey	5	Cow	99.30
10	Jersey	5	Cow	26.10
11	Jersey	5	Cow	57.30
12	Jersey	2	Heifer	53.17
13	Holstein-Friesian	6	Cow	150.15
14	Holstein-Friesian	5	Cow	207.82
15	Holstein-Friesian	8	Cow	199.69
16	Holstein-Friesian	7	Cow	222.68
17	Holstein-Friesian	3	Cow	160.83
18	Hereford	1	Bull	149.08
Average				108.09
Jersey average				58.16
Holstein-Friesian average				170.50

utilizes the auto analyzer. Samples were read at 480 mμ in the spectrophotometer and results expressed in mg./100 ml.

Blood pH

Blood samples for pH determinations were collected from the coccygeal vein into a 1 ml. plastic syringe containing sodium heparin as the anticoagulant. Immediately, the samples were placed in an ice bath and values determined by using Micro Electrode Unit E5021* as described by Andersen *et al.* (1968).

Since blood pH was to be an important phase of studies on ammonia toxicosis, 10 Jersey cattle were used to determine normal values and to find out if these values would be affected by a change from drylot to brome grass and orchard grass pasture. These cattle were bled, exposed to pasture, and rebled as previously described in the plasma ammonia nitrogen determinations.

To compare the effects of delayed versus immediate determination on blood pH value, 2 Guernsey and 4 Holstein-Friesian cattle were bled and sampled immediately. The samples were then maintained at 25 C. and read at 10, 20 and 60 minute intervals.

Serum Electrolytes

Blood was collected from the jugular vein into 7 ml. vacutainer vials for serum electrolyte determinations (magnesium, phosphorus, calcium, chloride, sodium and potassium). The serum was transferred into a 3 ml. glass vial and refrigerated at -4 C. overnight. The serum sample was then thawed at 25 C. and analyzed in the laboratory.

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*Radometer, Copenhagen, Denmark.

Magnesium. Serum magnesium was determined by the microtechnique of Bohuon (1962). Harleco #25831* was used as the standard. The samples were read in a spectrophotometer at 510 mμ and the results recorded in mEq./L.

Phosphorus. Blood serum samples were analyzed by Hycel procedures** with potassium phosphate as the standard. The unknown and standard were spectrophotometrically read at 650 mμ. To form a standard curve, the optical densities of the standard were plotted against concentration on regular graph paper. These results were recorded in mg./100 ml.

Calcium. Serum calcium was determined by the titration method of Backra *et al.* (1958) with the titrations made from a known calcium standard. The following formula was used to obtain values which were then reported in mg./100 ml.

$$\text{ml. EDTA (titrate unknown)} \times \frac{\text{Concentration Standard}}{\text{ml. EDTA (standard)}}$$

$$= \text{Concentration of calcium in sample}$$

Chloride. Serum chloride was analyzed by the method of Schales and Schales (1941). Sodium chloride was used as the standard. The following formula was used and results reported in mEq./L.

$$\frac{\text{Chloride Conc. of Unknown}}{\text{No. ml. } Hg(NO_3)_2 \text{ used to titrate unknown}} = \frac{\text{Conc. of Standard}}{\text{No. of ml. } Hg(NO_3)_2 \text{ used to titrate standard}} = \text{Concentration of chloride in sample}$$

*Hartman-Leddon Company, Inc., Philadelphia, Pennsylvania.

**Hycel, Incorporated, P. O. Box 36329, Houston, Texas.

Sodium and Potassium. Sodium and potassium were determined in blood serum by using the Model 21 Coleman Flame Photometer* (Coleman Model 21 Flame Photometer D-248A 1967 Operation Manual). Sterox reagent** was used for both determinations with known sodium and potassium solutions as standards. Results were automatically determined on a spectrophotometer and expressed in mEq./L.

To determine if brome grass and orchard grass pasture would affect the serum electrolyte values of drylot cattle, 10 Jersey cattle were initially bled. Five of the cattle were turned out on pasture and 5 cattle were maintained in the drylot. After 3 days all cattle were rebled and pasture versus drylot values were compared.

Experimental Ammonia Toxicosis

Experimental Animals. Two Holstein-Friesian steers, 14 months of age, were used in this research. Both animals were housed in a single 10 x 10 pen. Alfalfa hay was fed *ad libitum*.

Preparation of Experimental Ammonium Compounds. The following freshly prepared solutions were used in individual experiments: ammonium carbonate (1.6%, 4%, 6%, 7%, 8%), ammonium chloride (7%) and ammonium oxalate (8%). These were prepared by mixing the ammonium compound** with 500 ml. of distilled water (Table 2). These compounds were injected intravenously to determine their effects on blood pH, serum electrolytes (chloride, sodium, potassium), blood urea nitrogen and plasma ammonia nitrogen levels.

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*Coleman Instruments, Incorporated, Maywood, Illinois.

**J. T. Baker Chemical Company, Phillipsburg, N.J.

Table 2. Identification and weight of experimental Holstein-Friesian steers, duration of treatments, ammonium treatments, dates of injections

Animal No.	Weight (kg.)	Duration of Injection (min.)	Ammonium Compound* (%)	Date of Injection (1968)
1	325	55	1.6 ammonium carbonate	8-1
1	338	42	4.0 ammonium carbonate	8-19
2	346	55	6.0 ammonium carbonate	8-20
2	328	58	7.0 ammonium carbonate	8-21
1	339	60	8.0 ammonium carbonate	8-22
2	338	18	8.0 ammonium chloride	9-2
1	347	17	7.0 ammonium oxalate	9-3**

*Volume was 500 ml. for each injection.

**Died.

Table 3. Weight of Holstein-Friesian steer No. 2, duration of treatment, urea levels, method of treatment, dates of treatment

Weight (kg.)	Duration of Treatment (min.)	Urea Levels (Gm.)	Method of Administration	Date of Treatment (1968)
355	85	158	Mixed in feed	10-11
379	120*	272	Mixed in feed and pumped in rumen	10-25

*Died.

Ammonium Compound Injection Technique. A 14 Ga. 2 inch needle was inserted into the jugular vein and the vein cannulated with polyethylene tubing No. 90.* A 20 Ga. 1 inch needle was inserted into the tubing and connected to a gravity flow intravenous outfit which had previously been connected to the plastic bottle containing the ammonium mixture. During the ammonium chloride and ammonium oxalate injections, an 18 Ga. 1-1/2 inch needle was used in place of the 20 Ga. 1 inch needle. There were a total of 7 experimental injections in which steer No. 1 was injected 4 times and steer No. 2 injected 3 times. Steer No. 1 died during the infusion of 7% ammonium oxalate.

Blood pH. To determine the effect of ammonium compounds on blood pH, steer No. 1 was injected intravenously with 4% ammonium carbonate and 7% ammonium oxalate and steer No. 2 injected intravenously with 6% ammonium carbonate.

Serum Electrolytes. Injections of 4% ammonium carbonate and 8% ammonium carbonate were administered intravenously to steer No. 1 to determine their effect on initial serum electrolyte values (chloride, sodium, potassium).

Blood Urea Nitrogen. To assess the influence of ammonium carbonate on blood urea nitrogen, steer No. 1 was injected intravenously with the following solutions: 1.6% ammonium carbonate, 4% ammonium carbonate and 8% ammonium carbonate.

Plasma Ammonia Nitrogen. Plasma ammonia nitrogen levels were an important phase in the experimentally produced ammonia toxicosis. These

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*Clay Allen, Incorporated, New York, N.Y.

levels could serve as a diagnostic aid in suspected toxicosis from ammonia producing compounds. To establish the influence on plasma ammonia nitrogen levels, steer No. 1 was injected intravenously with 1.6% ammonium carbonate, 4% ammonium carbonate, 8% ammonium carbonate and 7% ammonium oxalate. Steer No. 2 was similarly injected with 6% ammonium carbonate, 7% ammonium carbonate and 8% ammonium chloride.

Urea Experiments

To terminate the investigation and compare the effects of ammonium compounds and urea, steer No. 2 was treated orally with 2 levels of feed-grade urea* (Table 3). Collection of blood and laboratory determinations were the same as previously described. The effects of urea on blood pH, blood urea nitrogen and plasma ammonia nitrogen levels were studied and compared with levels obtained following earlier intravenous injections of ammonium compounds. For a period of 12 days prior to the first treatment, steer No. 2 was fed a diet of 1150 Gm. of corn, 1150 Gm. of oats, 916 Gm. of molasses and alfalfa hay was fed *ad libitum*. Twenty-four hours before treatment, the diet was halved. On the morning of the treatment, 158 Gm. of urea was added to the whole diet and fed to the steer. All feed was consumed. The same diet was fed for a similar period prior to the second treatment. Eighteen hours before this treatment all water was withheld. On the morning of the treatment, 286 Gm. of 44% soybean oil meal and 250 Gm. of urea were added to the whole diet and fed to the steer. After a 15-minute interval, the steer refused to eat. At that time, 166 Gm. of urea was mixed with 2 liters of tap water and administered by stomach pump through a stomach tube

*Eastman Organical Chemicals, Rochester, N.Y.

into the rumen. All feed not eaten by the steer was weighed to determine the total urea intake. Before death, a total of 272 Gm. of urea had been administered.

Necropsy

Following the death of both steers, a necropsy was performed. Organs examined were kidneys, cerebellum, spinal cord, heart, abomasum and liver. Tissues were fixed in 10% buffered formalin, processed routinely and paraffin sections were cut at 6 mμ and stained by the hematoxylin-eosin method (Armed Forces Institute of Pathology *Manual of Histologic and Special Staining Technics*, 1957).

RESULTS

When whole heparinized blood samples were collected from Jersey cattle and maintained at 25 C. and analyzed at various time intervals, there was a marked decrease in blood plasma ammonia nitrogen levels (Tables 4, 5 and 6). The data indicated that testing or analyzing for blood plasma ammonia nitrogen levels should not be delayed longer than 30 minutes after the initial collection, to ensure accuracy.

Pasturing Jersey cattle on brome grass and orchard grass for a period of 3 days caused a marked increase in blood plasma ammonia nitrogen levels (Table 7). The results indicated that diet can affect blood plasma ammonia nitrogen levels. Blood pH values were recorded in 10 Jersey cattle (Table 8). The results indicated that the normal blood pH values of these cattle was 7.32. The effects of access to pasture versus maintenance in a drylot are illustrated (Table 8). The results indicated there was only a minimal effect on blood pH. Delaying the readings for blood pH until 10, 20 or ~~30~~ 60 minutes after the initial reading caused only slight alteration from initial blood pH values in Guernsey and Holstein-Friesian cattle (Table 9).

The effects of access to brome grass and orchard grass pasture on the blood serum magnesium, phosphorus, calcium, chloride, sodium and potassium levels of Jersey cattle are shown (Table 10). The results indicated that the brome grass and orchard grass pasture caused only minimal changes from the initial values of these blood serum electrolytes.

Table 4. Effect of delayed reading time on blood plasma ammonia nitrogen levels in Jersey cattle

Animal No.	Initial Sample (µg./100 ml.)	27 Minute Time* Interval (µg./100 ml.)
4	43.80	34.95
7	33.00	30.30
8	26.70	20.50
10	27.90	18.90
12	44.55	31.35
Average	35.19	27.20

*Paraffin placed on sample after initial reading.

Table 5. Effect of delayed reading time on blood plasma ammonia nitrogen levels

Animal No.	Initial Sample (µg./100 ml.)	Time Interval 60 (min.) (µg./100 ml.)	Time Interval 18* (hr.) (µg./100 ml.)
5	63.30	63.30	49.20
9	99.30	91.20	73.80
11	57.30	57.30	47.85
Average	73.30	70.26	56.95

*Paraffin placed on sample after reading and sample refrigerated at 7 C.

Table 6. Effect of delayed reading time on blood plasma ammonia nitrogen levels

Animal No.	Initial Sample (µg./100 ml.)	Minute Time Interval 10* (µg./100 ml.)	30* (µg./100 ml.)	40** (µg./100 ml.)
6	132.60	128.85	124.20	120.90
8	79.20	73.95	67.95	63.15
10	24.30	18.75	18.75	15.00
12	61.80	58.50	53.85	51.45
Average	74.45	70.01	66.18	62.62

*Paraffin placed on sample after initial reading.

**Paraffin removed from sample after second reading.

Table 7. Effect of brome grass and orchard grass pasture on blood plasma ammonia nitrogen levels in Jersey cattle

Animal No.	Initial Sample (µg./100 ml.)	Drylot Sample (µg./100 ml.)	Pasture Sample (µg./100 ml.)
3	28.10	79.35	---
4	43.80	74.40	---
5	63.30	82.50	---
6	132.60	64.05	---
8	52.95	---	219.75
9	99.30	---	166.65
10	26.10	---	234.60
11	57.30	---	160.20
12	53.17	---	125.55
Average	61.84	75.07	181.35

Table 8. Effect of brome grass and orchard grass pasture on blood pH of Jersey cattle

Animal No.	Initial Sample	Drylot Sample	Pasture Sample
3	7.26	---*	---
4	7.36	7.23	---
5	7.30	7.23	---
6	7.31	7.30	---
7	7.31	7.38	---
8	7.32	---	7.40
9	7.38	---	7.40
10	7.32	---	7.35
11	7.38	---	7.35
12	7.30	---	7.14
Average	7.32	7.28	7.29

*Escaped from drylot.

Table 9. Effect of delayed reading on blood pH in Guernsey and Holstein-Friesian cattle

Animal No.	Breed	Initial Sample	Time Interval in Minutes 10	20	60
19	Guernsey	7.46	7.45	7.47	7.47
20	Guernsey	7.36	7.34	7.33	7.35
21	Holstein-Friesian	7.36	7.34	7.35	7.37
22	Holstein-Friesian	7.36	7.36	7.33	7.35
23	Holstein-Friesian	7.44	7.38	7.38	7.39
24	Holstein-Friesian	7.45	7.34	7.42	7.44
Average		7.40	7.38	7.38	7.39

Table 10. Effect of exposure to brome grass and orchard grass pasture on blood serum magnesium, phosphorus, calcium, chloride, sodium and potassium levels of Jersey cattle

Animal No.	Age (yr.)	Magnesium (mEq./L)	Phosphorus (mg./100 ml.)	Calcium (mg./100 ml.)	Chloride (mEq./L)	Sodium (mEq./L)	Potassium (mEq./L)
				Initial Sample			
3	7	2.3	2.0*	9.6	114	137	4.3
4	6	2.7	7.4	9.6	106	139	4.8
5	4	2.9	7.3	10.3	110	142	4.0
6	3	2.8	3.8**	9.6	111	141	3.7
7	3	2.5	7.2	10.5	106	139	4.6
8	1	2.5	7.8	10.5	105	136	4.4
9	5	2.1	6.9	10.0	106	137	4.2
10	5	2.5	5.5	10.1	103	137	4.5
11	5	2.3	5.1	10.3	104	135	4.2
12	2	2.7	4.9	8.9	114	141	4.6
Average		2.5	5.7	9.9	107	138	4.3
				Yard Sample--3 Days			
3*	7	---	---	---	---	---	---
4	6	2.9	5.8	10.5	99	140	4.1
5	4	2.8	7.0	10.7	103	132	4.4
6	3	2.5	7.3	9.4	103	140	4.7
7	3	3.2	6.6	9.9	109	138	4.2
Average		2.8	6.6	10.2	103	137	4.3
				Pasture Sample--3 Days			
8	1	2.9	6.6	9.9	103	133	4.3
9	5	3.0	3.8	11.5	107	131	4.3
10	5	2.3	5.8	9.4	103	137	4.1
11	5	2.8	5.1	9.9	105	130	3.7
12	2	2.7	4.9	10.5	107	134	4.1
Average		2.7	5.2	10.6	105	133	4.1

*Crippled
**Calved recently

The results of the intravenous infusion of steer No. 1 with 4% ammonium carbonate and 7% ammonium oxalate and steer No. 2 with 6% ammonium carbonate and their effects on blood pH are illustrated (Table 11). The data indicated that these ammonium compounds caused only a slight elevation of blood pH values.

The effects of the intravenous injections of 4% ammonium carbonate and 8% ammonium carbonate on the blood serum chloride, sodium and potassium levels of steer No. 1 are recorded (Table 12). Ammonium carbonate had only a minimal effect on blood serum chloride, sodium and potassium.

The results of the intravenous infusions of 1.6% ammonium carbonate, 4% ammonium carbonate and 8% ammonium carbonate on the blood urea nitrogen levels of steer No. 1 are illustrated (Table 13). Treatment with ammonium carbonate resulted in blood urea nitrogen levels which were essentially the same as the initial levels.

The effects on blood plasma ammonia nitrogen levels following the intravenous infusions of steer No. 1 with 1.6% ammonium carbonate, 4% ammonium carbonate, 8% ammonium carbonate and 7% ammonium oxalate and steer No. 2 with 6% ammonium carbonate, 7% ammonium carbonate and 8% ammonium chloride are recorded (Table 14). The results indicated these ammonium compounds caused a marked increase in plasma ammonia nitrogen levels which were directly proportional to the percentage of ammonium compound injected. Steer No. 1 died following the intravenous infusion of 7% ammonium oxalate. Blood plasma ammonia nitrogen levels were a reliable indication of the severity of ammonia toxicosis.

The effects of the administration of 158 Gm. and 272 Gm. of urea on blood pH values of steer No. 2 are illustrated (Table 15). Both levels of urea caused a slight elevation of the initial blood pH values.

Table 11. Effect of the intravenous injection of 500 milliliters of 4% ammonium carbonate, 6% ammonium carbonate, and 7% ammonium oxalate on the blood pH of Holstein-Friesian steers

Animal No.	Weight (kg.)	Ammonium Compound (%)	Duration of Infusion (min.)	Shortly Before Infusion	Immediately After Infusion
1	338	4% ammonium carbonate	42	7.34	7.40
2	346	6% ammonium carbonate	55	7.30	7.35
1	347	7% ammonium oxalate	17	7.30	7.32*
Average				7.31	7.35

*Died.

Table 12. Effect of the intravenous injection of 500 milliliters of 4% ammonium carbonate and 8% ammonium carbonate on the blood serum chloride, sodium and potassium levels in Holstein-Friesian steer No. 1

Date of Treatment (1968)	Weight (kg.)	Duration of Infusion (min.)	Ammonium Carbonate (%)	Initial Sample			Postinjection Sample		
				Chloride (mEq./L)	Sodium	Potassium	Chloride (mEq./L)	Sodium	Potassium
8-19	338	42	4	99	138	4.3	104	112	5.0
8-22	339	60	8	102	141	4.1	92	143	3.9

Table 13. Effect of intravenous injection of 500 milliliters of 1.6% ammonium carbonate, 4% ammonium carbonate, and 8% ammonium carbonate on the blood urea nitrogen levels in Holstein-Friesian steer No. 1

Date of Injection (1968)	Injection Time (min.)	Weight (kg.)	Ammonium Carbonate (%)	Initial Sample (mg./100 ml.)	Postinjection Sample (mg./100 ml.)
8-1	55	325	1.6	16	16
8-19	42	338	4	4	6
8-22	60	339	8	19.5	20

Table 14. Effect of intravenous injection of 500 milliliters of 1.6% ammonium carbonate, 4% ammonium carbonate, 6% ammonium carbonate, 7% ammonium carbonate, 8% ammonium carbonate, 8% ammonium chloride and 7% ammonium oxalate on blood plasma ammonia nitrogen levels in Holstein-Friesian steers

Animal No.	Infusion Time (min.)	Infusion Date (1968)	Ammonium Compound (%)	Initial Sample (µg./100 ml.)	Postinfusion Sample (µg./100 ml.)
1	55	8-1	1.6 ammonium carbonate	148.20	278.80
1	42	8-19	4.0 ammonium carbonate	141.90	445.20
2	55	8-20	6.0 ammonium carbonate	126.00	732.00
2	58	8-21	7.0 ammonium carbonate	208.50	1205.70
1	60	8-22	8.0 ammonium carbonate	190.35	1319.40
2	18	9-2	8.0 ammonium chloride	---	1484.70
1	17	9-3	7.0 ammonium oxalate	---	1507.50*

*Died

Table 15. Effect of the administration of urea on the blood pH of Holstein-Friesian steer No. 2

Date of Administration (1968)	Weight (kg.)	Urea Level (Gm.)	Duration of Treatment (min.)	Initial Sample	Sampling Interval in Minutes 60	85	120
10-11	355	158	85	7.15	---	7.30	---
10-25	379	272	120	7.29	7.30	---	6.92*

*Died

Table 16. Effect of urea on the blood urea nitrogen level of Holstein-Friesian steer No. 2

Duration of Treatment (min.)	Weight (kg.)	Urea Level (Gm.)	Initial Sample (mg./100 ml.)	Time Interval in Minutes Postinjection Sample (mg./100 ml.) 60	85	120
85	355	158	9	---	18	---
120	379	272	11	29*	---	31**

*Paralyzed

**Died

Death resulted after the administration of 272 Gm. of urea. Blood pH decreased just prior to death of steer No. 2.

Blood urea nitrogen levels of steer No. 2 were changed at 60, 85 and 120 minutes as a result of feeding 158 Gm. and 272 Gm. of urea (Table 16). The data indicated that feeding of these levels of urea will cause a marked increase in blood urea nitrogen.

The effects on blood plasma ammonia nitrogen levels due to the oral administration of 158 Gm. and 272 Gm. of urea to steer No. 2 are recorded (Table 17). The results indicated that high levels of urea caused a marked increase in initial blood plasma ammonia nitrogen levels.

Clinically ammonium compounds and urea produced essentially the same toxic signs. These signs were rumen atony, muscle tremors, frequent urination, shallow respiration, ataxia, tachycardia, tetany, clonic convulsions, paralysis and death. The signs in Holstein-Friesian steer No. 2 following the administration of 272 Gm. of urea are shown (Figures 1, 2, 3 and 4).

Characteristic gross or microscopic lesions were not found in steer No. 1 that died as a result of 7% ammonium oxalate. Marginal perivascular and perineuronal edema in all sections of the central nervous system and edema of the lamina propria of the abomasal wall was present on necropsy of steer No. 2 that died from a high level of urea.

Table 17. Effect of urea on the blood plasma ammonia nitrogen level in Holstein-Friesian steer No. 2

Date of Treatment (1968)	Weight (kg.)	Duration of Treatment (min.)	Method of Administration	Urea Level (Gm.)	Initial Level (µg./100 ml.)	Time Interval in Minutes 60 (µg./100 ml.)	85	120
10-11	355	85	Mixed in feed	158	164.01	---	640.14	---
10-25	379	120	Mixed in feed and pumped into the rumen	272	137.25	2156.10	---	3428.40*

*Died

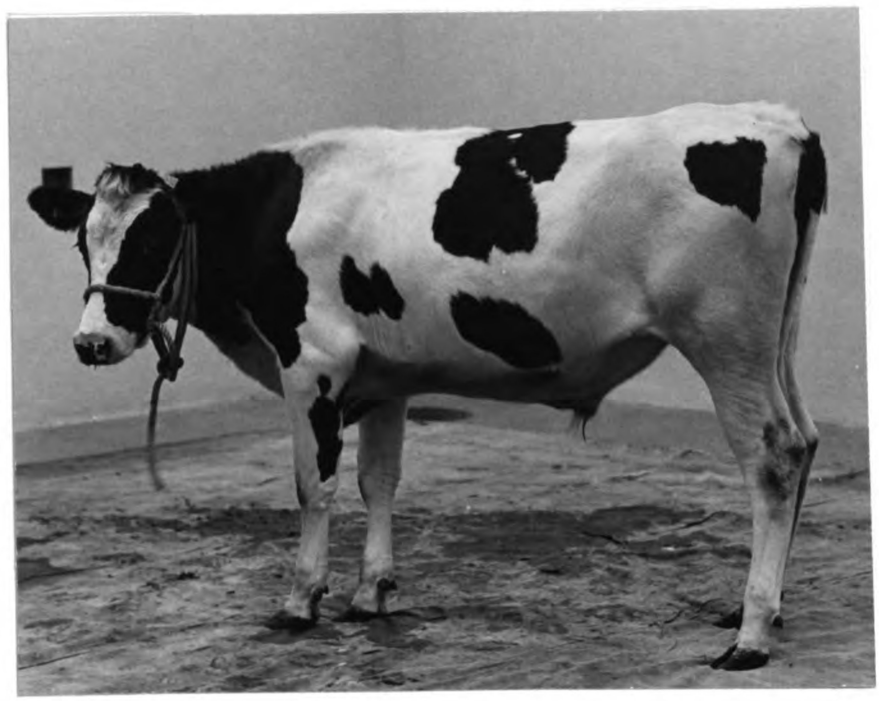

Figure 1. Holstein-Friesian steer No. 2, 15 minutes after administration of 272 Gm. of urea.

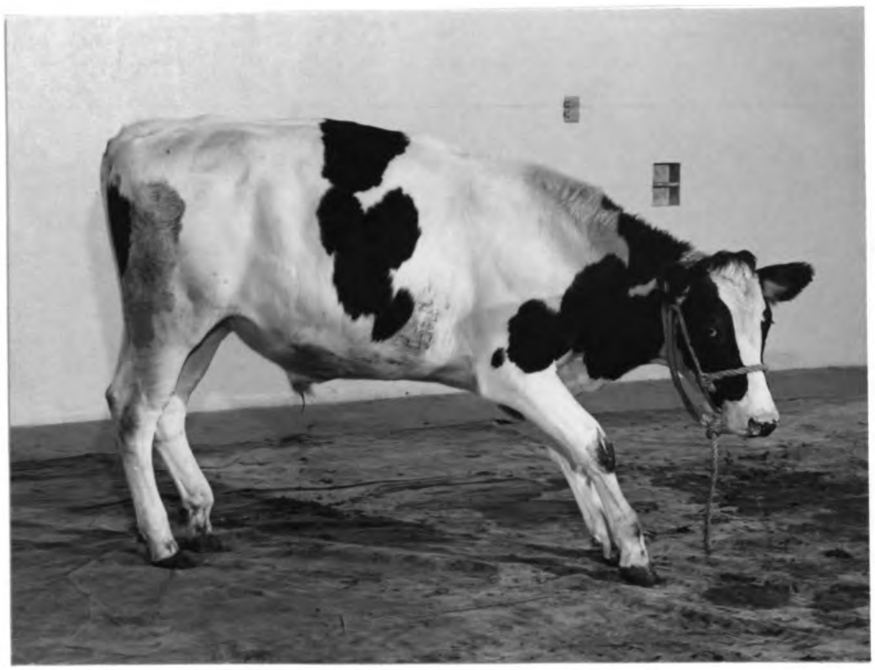

Figure 2. Initial urea toxicosis in Holstein-Friesian steer No. 2, 30 minutes after administration of 272 Gm. of urea.

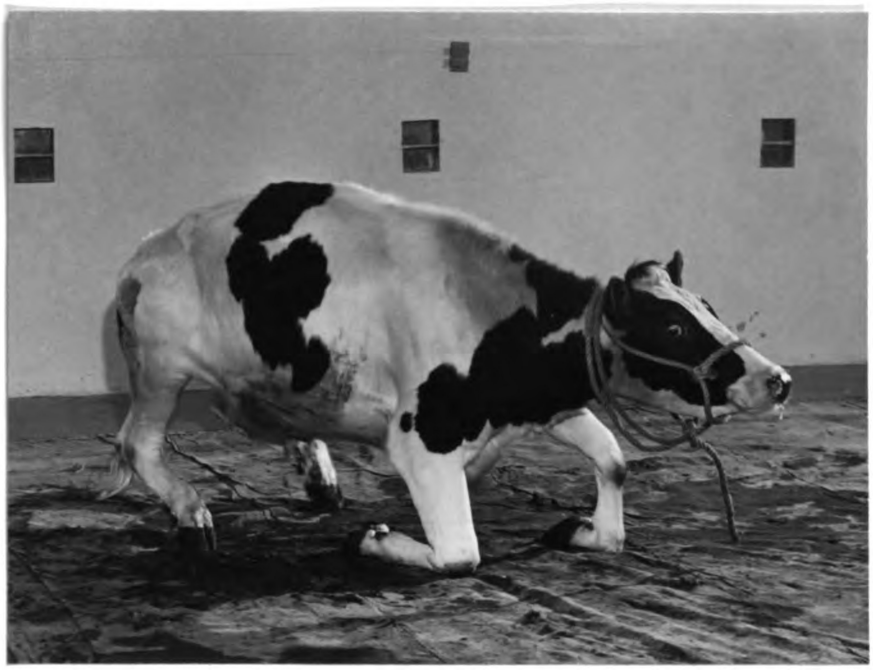

Figure 3. Urea toxicosis. Increasing toxic signs 45 minutes after administration of 272 Gm. of urea to Holstein-Friesian steer No. 2.

Figure 4. Urea toxicosis. Paralysis prior to death of Holstein-Friesian steer No. 2 following administration of 272 Gm. of urea. Sixty minutes after administration.

DISCUSSION

This research was conducted to clinically evaluate ammonia toxicosis in Holstein-Friesian steers. The data indicated that ammonium compounds injected intravenously or urea administered orally could result in toxicosis and death in Holstein-Friesian steers. The research further indicated that the severity of the toxicosis was dependent on the percentage of ammonium compound injected and the level of urea administered. The physiological effects were compared.

Ammonium compounds and urea produced essentially the same toxic signs. The signs observed were rumen atony, muscle tremors, polyuria, shallow respiration, ataxia, tachycardia, tetany, clonic convulsions, paralysis and death. These signs were similar to those reported in cattle (Whitehair, 1961), in pigs (Bicknell, 1966) and in dogs and sheep (Wilson *et al.*, 1968). Additional toxic signs observed in the urea experiments were flexion of the pasterns, extension of the fore and hind limbs and apparent blindness.

As the toxic signs were essentially the same following the intravenous injections of ammonium salts of carbonate, chloride and oxalate and administration of urea into the rumen this research suggests that the ammonium ion was the cause of toxicosis. This had been suggested in ammonia toxicosis studies in sheep (Lewis, 1960; Wilson *et al.*, 1968) and in pigs (Bicknell, 1966) and in dogs (Wilson *et al.*, 1968). These results did not confirm the conclusions of Kaishio *et al.* (1951) and Hale and King (1955) in sheep that ammonium carbamate was the toxic

intermediate. Lewis (1960) questioned the stability of ammonium carbamate and Wilson *et al.* (1968) were of the opinion that had these authors used ammonium carbonate in place of ammonium carbamate the same toxic effects would have resulted. Since this research involved the intravenous injections of 3 ammonium compounds, the carbamate ion would not be formed since the rumen was bypassed, therefore lending support to the theory that the ammonium ion is the cause of toxicosis.

Salivation was observed in lambs and steers following administration of 20 Gm. of urea per 40 kg. body weight (Dinning *et al.*, 1948) but was not observed in my research. This could be due to a difference in diet.

Rumen atony was the first clinical sign of ammonia toxicosis. The degree of rumen atony was dependent on the percentage of ammonium compound injected. At the highest percentage, atony was complete in 5-10 minutes after the injection was started and remained during the injection. Normal rumen contractions returned within 17 to 25 minutes after the injections ceased. This would indicate that the ammonium ion has only a temporary effect on rumen contractions under such experimental procedures. Further research should be conducted to determine the cause of this atony.

Urea administered to Holstein-Friesian steer No. 2 resulted in rumen atony within 22 minutes following the administration, which would agree with similar work in sheep (Annicolas *et al.*, 1956). In steer No. 2, which died from a high level of urea, the rumen pH increased above 8. This increased pH was in agreement with urea experiments in sheep (Coomber and Tribe, 1958). Hogan (1961), in similar experiments, indicated that as the pH increases in the rumen there is an increased transfer of ammonia across the rumen epithelium. To decrease pH may be

the rationale for using acetic acid to treat urea toxicosis.

Bloating as reported in consumption of high levels of urea in sheep (Gallup *et al.*, 1953) and in dairy cattle (Bullington *et al.*, 1955) was not observed in this research. This indicated that other factors than rumen atony were necessary to cause bloating or there was a difference in diets fed.

Muscle tremors were an early manifestation of ammonia toxicosis and initially involved the triceps muscles. The severity of the tremors and the involvement of the skeletal musculature were directly proportional to the percentage of ammonium compound intravenously injected or the level of urea administered orally. Following urination there was a temporary decrease in muscle tremors. As tremors were the first external sign of ammonia toxicosis, they would be a good clinical diagnostic aid as to the severity of the ammonia toxicosis.

Shallow respirations were observed as a clinical sign of ammonia toxicosis. In both steers that died, the respiratory centers were paralyzed prior to death. The increased respiration was possibly an attempt on the steer's metabolic system to eliminate excess hydrogen ions.

The increase in heart rate was directly proportional to the percentage of ammonium compound injected or the level of urea administered. The marked tachycardia and ventricular fibrillation prior to death indicated that the ammonium ion had a direct cardiotoxic effect. This was suggested by Lewis (1960) in urea experiments in sheep. Similar conclusions were indicated by Wilson *et al.* (1968) following the intravenous injection of ammonium carbamate in a decerebrated and bilaterally vagotomized dog.

Polyuria occurred in the ammonia toxicosis experiments, and the frequency was directly proportional to the percentage of ammonium compounds injected or level of urea administered. The temporary decrease in muscle tremors following urination has not been reported in the literature and was an outstanding finding in this research. Certain ammonium compounds have a diuretic action due to their osmotic effect on the body electrolytes. Urea, which is poorly reabsorbed from the tubules of the kidneys, attracts water from the body and thus has a diuretic action. This temporary decrease in muscle tremors following urination would provide a good problem for additional research in ammonia toxicosis.

The blood plasma ammonia nitrogen levels were a good indication of the degree of ammonia toxicosis. These results were in agreement with ammonia toxicosis studies in sheep (Lewis, 1960). Death occurred when the peripheral blood levels were between 1.4 and 3.2 mg./100 ml. The levels were essentially the same as reported in sheep (Lewis, 1960) and in protein experiments in dairy cattle (Holzachuh and Wetterau, 1962). If procedures are properly carried out, blood plasma ammonia nitrogen levels would serve as a diagnostic aid in suspected cases of ammonia toxicosis. The results indicated that to ensure accuracy, the plasma sample must be immediately analyzed following completion of the test procedures.

Ammonium compounds had little effect on blood urea nitrogen levels, whereas urea caused a marked increase. As these levels were compatible with life, the results indicated that blood urea nitrogen levels were not a good indication of the severity of ammonia toxicosis. Davis and Roberts (1954), in their urea experiments in cattle, indicated blood urea nitrogen levels of 22 mg./100 ml. caused no toxic effects, while

death and toxicosis occurred when the levels reached 42 mg./100 ml. The increase in blood urea nitrogen following the administration of urea would indicate that all urea was not hydrolyzed and excess urea diffused across the rumen wall. This is in agreement with Dinning *et al.* (1948) in urea experiments in sheep and cattle.

The absence of characteristic gross and microscopic lesions in steer No. 1 that died from ammonium oxalate injection indicated that this compound produced an acute toxicosis. Frequently in an acute toxicosis lesions are absent. The findings in this experiment were in agreement with ammonia toxicosis in pigs (Bicknell, 1966). In steer No. 2 that died from the administration of urea, the diffuse hemorrhages on the mucosal surface of the abomasum were due to the irritating properties of excess urea. Essentially the same findings were reported in the urea pathology in ruminants (Fujimoto and Tajima, 1953). The ecchymotic hemorrhages noted on the endocardium and myocardium were in agreement with similar lesions reported by Clark *et al.* (1951) in urea toxicosis studies in sheep.

Histologically both steers had a toxic nephrosis which indicated that the excessive urea eliminated by the kidneys was causing degenerative changes. In steer No. 2 the perivascular and perineuronal edema in all sections of the central nervous system could be possibly correlated with the convulsions noted in this animal prior to death.

In this research, the steers either died within 2 hours or recovered. During the course of the experimental procedures little effect was noted on their appetite or their weight gain. These results indicated that ammonium compounds or urea did not have a permanent effect on surviving animals.

The minimal effect on blood pH from administration of urea or injection of ammonium compounds indicated that the acid base balance of the blood was not a factor in ammonia toxicosis. Similar findings were noted on blood serum chloride, sodium and potassium following the injection of an ammonium compound.

Additional Procedures

These procedures were not a part of my research; however, during the experimental procedures additional procedures were carried out.

The results indicated in reading blood pH immediate determinations are not necessary to ensure accuracy.

In the brome grass and orchard grass pasture determinations, the results indicated that these grasses did not have an effect on blood pH.

Initial serum electrolyte levels were not affected by the intravenous injection of ammonium carbonate, indicating that serum electrolytes are not altered in ammonia toxicosis. Serum electrolytes were not affected by exposure to brome grass and orchard grass pasture and therefore these grasses are probably not a factor in regard to calcium, phosphorus and magnesium in muscle tetany in conditions associated with lush spring pasture.

Jersey cattle exposed to brome grass and orchard grass pasture resulted in a marked increase in blood plasma ammonia nitrogen levels over those maintained in drylot. These levels were not much higher than levels determined in other cattle. These results indicated that the pasture grass was rich in protein content.

SUMMARY

Toxicosis and death were produced in Holstein-Friesian steers by the intravenous injections of ammonium compounds and the administration of urea into the rumen. The severity of the toxicosis was directly proportional to the level of compound given.

When the peripheral blood plasma ammonia nitrogen levels reached 0.7 mg./100 ml., toxicosis was noted and death occurred when levels were between 1.5 and 3.2 mg./100 ml.

Signs of toxicosis were essentially the same for both the ammonium compounds and the urea. Toxic signs observed were ataxia, rumen atony, muscle tremors, polyuria, shallow respiration, tachycardia, paralysis of the Holstein-Friesian steers, convulsions and death. Additional toxic signs noted with high urea levels were flexion of the pasterns, extension of the legs and apparent blindness. There was a temporary decrease in muscle tremors following urination.

The Holstein-Friesian steers died following ventricular fibrillation which indicated that the ammonium ion had a direct toxic effect on the heart. Since similar toxic signs were caused by the administration of ammonium compounds and urea, it was concluded that the ammonium ion was the cause of death of the Holstein-Friesian steers and the cause of the syndrome known as urea toxicosis.

Twenty to thirty Grams of urea per 40 kg. of body weight will produce toxicosis and death in Holstein-Friesian steers. Following ingestion of toxic levels of urea, animals will usually become sick and die within

2 hours or recover. Intravenous injections of 0.8 mM/kg. of ammonium carbonate produced toxicosis; however, the animals survived, while 0.6 mM/kg. of ammonium oxalate was fatal.

On necropsy, no characteristic gross or microscopic lesions were observed in the steer that died from the intravenous injection of ammonium oxalate. Perivascular and perineuronal edema of the central nervous system, ecchymotic hemorrhages of the mucosa of the abomasum, and vacuolization of the chief cells of the cortex of the kidneys were present on necropsy of the steer that died from a high urea level.

Blood plasma ammonia nitrogen levels in Holstein-Friesian steers increased in direct proportion to the amount of ammonium compound injected or the amount of urea administered into the rumen. These levels could be used as a diagnostic aid in cases of suspected ammonia toxicosis from the overconsumption of urea. By maintaining plasma samples at 25 C. and analyzing at delayed time intervals there was a marked decrease in ammonia nitrogen levels, indicating the importance of reading samples immediately after completing the ammonia nitrogen test procedures.

Initial blood urea nitrogen levels of Holstein-Friesian steers were increased after the administration of ammonium compounds and urea. However, these levels were compatible with life and therefore were not a reliable indication of the severity of ammonia toxicosis.

Intravenous injections of ammonium compounds in Holstein-Friesian steers resulted in a blood pH increase. Similar effects were noted with urea administration initially; however, the blood pH decreased prior to death. Maintaining blood samples of Jersey cattle at 25 C. and analyzing at delayed time intervals caused little alteration of blood pH values. Pasturing of Jersey cattle on brome grass and

orchard grass pasture caused only a slight increase above initial values.

Injections of ammonium compounds had little effect on serum chloride, sodium and potassium levels of Holstein-Friesian steers. Jersey cattle were pastured on brome grass and orchard grass pasture for a period of 3 days with minimal increase in serum magnesium, phosphorus, calcium, chloride, sodium and potassium.

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VITA

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Following graduation the author worked for Dr. J. J. Lupfer, a general practitioner at Litchfield, Minnesota, until July 1945. In August 1945, he purchased a mixed practice from Dr. O. Osborn at Paynesville, Minnesota.

In February 1949 the author was called into the Armed Forces, Veterinary Corps, with the rank of Lieutenant and served in Chicago, Illinois. He was discharged in February 1950 and returned to Paynesville, Minnesota. The author was recalled into the Armed Forces, Veterinary Corps, in March 1951 with the rank of Captain. Duty stations were Germany and France. He was discharged in December 1952 and entered general practice with Dr. S. D. Sleight at Columbus, Wisconsin.

In July 1966, the author accepted a position on the faculty at Michigan State University with primary responsibilities to Ambulatory Clinic. At this time he also entered graduate school in the Department of Veterinary Surgery and Medicine at the university.