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Experimental ammonia poisoning in cattle fed extruded or prilled urea: clinical findings

Intoxicação experimental por amônia em bovinos que receberam uréia extrusada ou granulada: achados clínicos

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Abstract

Twelve yearling Girolando, rumen-fistulated steers never fed with urea before, were distributed randomly in 2 groups of 6 animals each. Both groups were administered intraruminally a single dose (0.5 g/kg BW) of extruded (G1) or prilled (G2) urea to induce ammonia poisoning. The clinical picture was followed for the next 240 min. Besides the classic signs the present study found 3 new additional sign: dehydration, hypothermia and ingurgitated episcleral veins. Convulsion, considered the definite sign, was seen in 5 out of 6 animals from both groups. One steer (G1) had only fasciculation, while another (G2) developed typical clinical signs, but not convulsion, and recovered spontaneously without treatment. The appearance of clinical signs such as muscle tremors, sternal recumbency and convulsive episode occurred at similar times in both groups, but when analyzed altogether they took place later in G1 ($p < 0.04$). The 1st sign to show up was fasciculation, followed by apathy, hyperaesthesia, pushing against obstacles, muscle tremor, rumen stasis, incoordination, sternal and then lateral recumbency, mild or severe dehydration, and convulsion. Higher heart rate was detected at the convulsive episodes. After the convulsions, 4 animals from each group had mild hypothermia. One steer from G2 fell down in coma and died suddenly before the beginning of the treatment. Although the extruded urea postponed the clinical picture, the signs were as severe as exhibited by cattle administered prilled urea. Both forms of urea offered at high dose can be harmful to cattle never fed urea.

Key-words:
Urea.
Ammonia poisoning.
Clinical picture.
Cattle.

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Introduction

Tropical grasses generally contain less crude protein than temperate grasses. The older the forage growth stage, the lower the crude protein content. Thus, cattle raised under extensive management on subtropical or tropical environment need to be supplemented with any source of protein or nitrogen, particularly during the dry season, in order to increase their productivity[1]. The cheapest way to supplement protein to cattle is through the use of non-protein nitrogen (NPN), mainly urea[2]. About 20 million Brazilian cattle are fed urea yearly, especially during the dry season[3].

The rumen microbiota hydrolyzes urea into ammonium (NH_4^+) and ammonia

(NH_3) to synthesize their own protein. Usually, most of the urea is converted to ammonium that is available to the rumen microbiota. Conversely, the small amount of ammonia produced, which is a lipid-soluble compound, is readily absorbed into the blood stream[2]. Normally, the liver can detoxify ammonia into urea efficiently, but at higher concentrations blood, ammonia will overwhelm hepatocytes capacity of detoxification, and will increase its levels in blood, cerebrospinal fluid and other tissues, resulting in ammonia poisoning.[4, 5] Animals used to high crude protein in the diet or adapted to urea have greater ability to detoxify ammonia in the liver.[2] While liver has this important role in ammonia detoxification, dysfunction of the organ leads to higher susceptibility to ammonia poisoning.[6]

Ammonia poisoning may occur periodically when ruminants gain access to large quantities or are fed large amounts of urea; when they are unadapted to it or when feeds are improperly mixed or a high urea concentration is present in low energy, low protein, and high roughage diets.[7] The onset of clinical signs may vary in a matter of a few minutes to hours after consumption of NPN and they are usually acute and drastic leading to death in most of the cases.[4,7] The clinical signs due to ammonia poisoning have been reviewed and described by several authors, but other studies showed that some clinical signs may not occur and others may be different.[2,8,9,10]

Trying to prevent ammonia poisoning an alternative supplement was developed, where urea was extruded with grain. Some authors stated that it prevents ammonia toxicity due to a slow release of the urea in the rumen.[9,11,12] Nevertheless, high dose of extruded urea may cause ammonia toxicity[11]. No studies have been carried out so far to compare the clinical picture of cattle poisoned with prilled and extruded urea. Although most of the Brazilian cattle is supplemented with prilled urea, the use of extruded urea is increasing progressively.

The purpose of this study was to register the main clinical picture presented by unadapted cattle to urea that were experimentally poisoned with high doses of prilled or extruded urea.

Materials and Methods

Twelve yearling girolando, rumen-fistulated, healthy steers (average weight of 200 kg) from a single herd were randomly divided in two groups of six animals each. They were housed indoors in individual tie stalls and were fed, for months before the trial, the following basal diet with 9.55% crude protein: 70% coast-cross hay (*Cynodon dactylon* (L) Pers) and 30% commercial concentrate. This diet did not contain urea or any source of NPN. Each animal was used only once in the trial. The Group 1 received 2.5 g/kg of body weight (BW) of extruded urea with 20% of urea and 80% of cornstarch; to the Group 2 it was given 0.5 g/kg BW of prilled urea and 2.0 g/kg BW of cornstarch, separately. Both groups received the equivalent amounts of urea and cornstarch.

The animals were fasted for 15 h preceding each induction. The urea and the cornstarch were administered within the ventral sac of the rumen with the help of a plastic tube through the fistula. Then, the rumen content was stirred by hand to assure complete spreading in the rumen. For the next 240 min the clinical picture exhibited was recorded. The heart and respiratory rates, ruminal movement, rectal temperature were recorded, and venous blood samples were collected in the following times: before giving urea, at the occurrence of the 1st clinical sign, muscle tremors, sternal recumbency, and at the 1st convulsive episode. Blood samples were collected from the jugular vein into tubes containing EDTA for the determination of hematocrit values.

When a convulsive episode was first observed or at the end of 240 min, the rumen contents were evacuated using a siphon, and

10 L of isotonic saline solution plus 4 L of rumen content from a healthy steer were administered through the fistula. Animals that exhibited severe poisoning with signs such as presence of multiple convulsive episodes and depressive mental state were treated intravenously with 1 mL/kg BW commercial solution of urea cycle amino acids (Ornitarginâ), 1mg/kg BW furosemide (Zalixâ), and 20 mL/kg BW isotonic saline solution.[3]

The data were assessed by ANOVA and the means compared by the Duncan's multiple range test. The coefficient of correlation was assessed between several variables.[13] The statistical analyses were performed using the MINITAB statistical software.[14]

Results

At the beginning of trials, the steers were healthy, alert and active. The first signs of poisoning occurred at the 56±17 and 41±11 min after the administration of urea for Groups 1 and 2, respectively (Table 1). The appearance of muscle tremors, sternal recumbency and convulsive episode occurred at similar time in both groups. When these signs were analyzed altogether they took place later in cattle that received extruded urea ($p < 0.04$) (Table 1).

The frequency of different clinical signs exhibited by cattle of both groups is shown in table 2. The first sign to show up in 75% of the animals was fasciculation, while for the remaining (25%) was apathy. The fasciculation began in the head, neck and front legs and then progressed to the trunk and hinds. One steer (Group 1) exhibited fasciculation and suddenly recovered. Another (Group 2) became apathetic, pushed against obstacles, had muscle tremor, rumen stasis and incoordination, laid down in sternal then lateral recumbency, but did not exhibit any convulsive episode, and recovered within the next 130 min, without any treatment. All remaining animals (n = 10) had various clinical signs (Table 2) that culminated with the presence of convulsive episodes.

Signs of behavioral changes were seen throughout the clinical picture. Six out of 11 steers showed initially uneasiness for 5 to 20 min and then all of them became apathetic until recovery, including the interspaces between the convulsions (Table 1). Despite the apathy, half of the steers had hyperaesthesia, particularly when touched or due to sudden noise. About 10 min before the muscle tremors started the animals were pushing against obstacles; many had mydriasis and exhibited ingurgitated episcleral veins. The muscular tremors started at 79±26 and 60±25 min in groups 1 and 2, respectively, and lasted 10 to 15 min. As soon as muscle tremors ended most of the steers salivated excessively, became uncoordinated, stiffened the forelimbs and laid down in sternal recumbency, followed by lateral recumbency. When the steers assumed this position, depression in the mental state and horizontal nystagmus were evident.

Ruminal motility decreased progressively throughout the clinical picture, and ceased completely when the animals were lying down in sternal recumbency. Despite the rumen stasis only two steers were bloated.

Table 1
Mean values and standard deviation of time after dosing urea to onset of clinical picture and main clinical signs of ammonia toxicity – Sao Paulo – 2002

Group	Onset (min)	Muscle tremors (min)	Sternal Recumbency (min)	Convulsion (min)	Overall mean (min)
1	[a] 56 ± 17	[a] 79 ± 26	[a] 94 ± 32	[a] 160 ± 66	[a] 95 ± 54
2	[a] 41 ± 11	[a] 60 ± 25	[a] 72 ± 32	[a] 95 ± 42	[b] 66 ± 33

Different superscripts in columns indicate significant differences ($p < 0.04$)

Table 2
Frequency of clinical signs recorded from experimentally induced ammonia poisoning due urea administration in steers, Sao Paulo, 2002

Clinical Signs	Group 1	Group 2
Uneasiness	2 / 6	4 / 6
Hyperaesthesia	2 / 6	4 / 6
Fasciculation	6 / 6	6 / 6
Muscle tremors	5 / 6	6 / 6
Apathy	5 / 6	6 / 6
Pushing against obstacles	5 / 6	6 / 6
Incoordination	5 / 6	6 / 6
Stiffening of the fore limbs	4 / 6	5 / 6
Sternal recumbency	5 / 6	6 / 6
Lateral recumbency	5 / 6	6 / 6
Rumen stasis	5 / 6	6 / 6
Bloat	1 / 6	1 / 6
Diarrhea	0 / 6	1 / 6
Profuse salivation	4 / 6	5 / 6
Dry muzzle	2 / 6	4 / 6
Enophthalmia	1 / 6	4 / 6
Mild dehydration (5%)	1 / 6	1 / 6
Severe dehydration (10%)	2 / 6	4 / 6
Pulmonary edema	2 / 6	4 / 6
Diuresis	2 / 6	2 / 6
Mydriasis	3 / 6	4 / 6
Ingurgitated episcleral veins	2 / 6	4 / 6
Horizontal nystagmus	4 / 6	5 / 6
Vocalization	0 / 6	1 / 6
Tachycardia	3 / 6	5 / 6
Depression in mental state	5 / 6	5 / 6
Convulsion	5 / 6	5 / 6
Hypothermia	4 / 6	4 / 6
Coma	0 / 6	1 / 6
Death	0 / 6	1 / 6

Many animals excreted soft stools, and one had diarrhea. Two animals from each group had diuresis. Signs indicative of mild or severe dehydration were present in many steers, such as lowered skin elasticity, dry muzzle and enophthalmia. Two animals from Group 1 and 4 from Group 2 had pulmonary edema, as seen by the presence of audible crackles on auscultation.

At the occurrence of the convulsive episodes the heart and respiratory rates and hematocrit values increased significantly in both Groups (Table 3). Usually the convulsions were short (30 to 40 sec) and interspaced by short quiescent periods (30 sec to 2 min). Most of the cattle had 4 or more convulsive episodes. The convulsion was characterized by stiffening of fore and hind limbs, and horizontal nystagmus; at the peak of the convulsion respiration stopped. Vocalization was recorded in a single steer (Group 2) at the convulsion. The rectal temperature decreased significantly after convulsive episodes in both groups (Table 3). The higher the heart rate, the lower the rectal temperature ($r = -0.65$). A positive correlation was found between hematocrit values and heart rate ($r = 0.73$), and a negative correlation between hematocrit

Table 3
Mean values and standard deviation of heart rate, respiratory rate, rumen movement, rectal temperature and hematocrit after dosing urea throughout the clinical picture of ammonia toxicity – Sao Paulo – 2002

	Group	Basal	Onset	Tremors	Sternal Recumbency	Convulsion
Heart Rate (bpm) *	1	$^{b}60 \pm 9$	$^{b}59 \pm 11$	$^{b}60 \pm 13$	$^{b}70 \pm 14$	$^{a}101 \pm 22$
	2	$^{b}56 \pm 7$	$^{b}59 \pm 6$	$^{b}59 \pm 8$	$^{b}62 \pm 10$	$^{a}92 \pm 17$
Respiratory Rate (mov/min) **	1	$^{b}17 \pm 3$	$^{b}17 \pm 5$	$^{b}16 \pm 3$	$^{b}18 \pm 5$	$^{a}25 \pm 3$
	2	$^{b}18 \pm 4$	$^{b}19 \pm 6$	$^{ab}20 \pm 8$	$^{ab}20 \pm 7$	$^{a}26 \pm 4$
Rumen Movement (mov/3 min) ***	1	$^{a}4 \pm 1$	$^{b}2 \pm 1$	$^{c}1 \pm 1$	$^{c}0$	$^{c}0$
	2	$^{a}3 \pm 1$	$^{b}2 \pm 1$	$^{c}0$	$^{c}0$	$^{c}0$
Rectal Temperature (°C) *	1	$^{a}38.2 \pm 0.4$	$^{a}38.3 \pm 0.4$	$^{a}38.3 \pm 0.4$	$^{a}38.5 \pm 0.6$	$^{b}36.8 \pm 1.0$
	2	$^{a}37.9 \pm 0.2$	$^{a}38.0 \pm 0.2$	$^{a}38.0 \pm 0.2$	$^{a}38.0 \pm 0.3$	$^{b}36.6 \pm 1.0$
Hematocrit (%) *	1	$^{b}33 \pm 3$	$^{b}33 \pm 3$	$^{b}35 \pm 2$	$^{ab}37 \pm 3$	$^{a}41 \pm 3$
	2	$^{b}35 \pm 4$	$^{b}35 \pm 4$	$^{ab}40 \pm 4$	$^{ab}41 \pm 3$	$^{a}45 \pm 4$

Different superscripts in lines indicate significant differences (* $p < 0.05$; ** $p < 0.04$; *** $p < 0.03$). There were no significant differences between groups

values and rectal temperature (r = -0.60), respectively.

Abdominal pain, characterized by kicking the abdominal wall, grinding of the teeth, hyperthermia and opisthotonus were never seen throughout the poisoning.

One steer from Group 2 fell down in coma right after the beginning of the convulsive episodes and died despite the treatment and intensive care. Mild pulmonary edema and lung congestion were the only gross lesions seen in the *post-mortem* exam.

The proposed treatment was successful in 91.7% of the cases. The clinical recovery in all cases was gradual and lasted 1 to 4 h. The first sign of recovery was the self-correction from lateral to sternal recumbency, followed by the return of rumen movements and appetite, standing up and driving themselves to their own stall.

Discussion

The dose of urea used to poison the cattle in both groups (0.5 g/kg BW) was more than twice of the standard dose normally offered to adapted cattle (0.22 g/kg BW). In order to increase the odds of poisoning, the steers were fed a low-protein ration and deprived of dietary urea. Thus, all animals exhibited the classical signs of ammonia poisoning, except one steer from the extruded-urea group. Both forms of urea caused ammonia poisoning in the steers, but extruded urea with grain delayed, in most cases, the onset of clinical signs, although it did not reduce the risks of ammonia poisoning and it was as severe as the one caused by prilled urea.

Although most of the clinical signs of acute ammonia poisoning described in this experiment (Table 1) have been cited by others[2,8,9,10], 3 new additional signs were observed: dehydration, hypothermia, and ingurgitated episcleral veins.

More than 50% of poisoned cattle presented severe dehydration confirmed by a significant increase in the hematocrit values and the presence of signs such as: enophthalmia, dry muzzle and lower skin elasticity. Ammonia *per se* is very irritative to the pulmonary tract and causes an intense migration of fluids to the lungs, resulting in lung edema that is a common finding in the necropsy of poisoned cattle[2,15]. All animals diagnosed as having lung edema (n = 6) had also severe dehydration. Thus, it is likely that part of dehydration might be caused by sequestering of fluids from the bloods to the lungs. Nevertheless, other two steers had mild dehydration without developing lung edema. More studies are necessary to fully understand the mechanism of fluid loss or migration due to ammonia poisoning.

To reduce the lung edema and to recover the hydration status the steers were treated intravenously with diuretic and large amounts of fluids, respectively. A recent study showed that this treatment associated with the use of urea cycle amino acids, to improve liver detoxification of ammonia, are efficient to recover from toxicity[3].

Mydriasis was seen in 7 steers. The pupilary aperture is controlled by the constrictor muscles, innervated by the oculomotor nerve (parasympathetic), and by the dilator muscles of the pupil (sympathetic). Mydriasis can occur whenever there is a parasympatholytic status or a sympathetic stimulation[16]. The presence of signs such as mydriasis and tachycardia suggests that ammonia poisoning could elicit a sympathetic or a parasympatholytic status. This hypothesis contradicts the one previously stated elsewhere that during ammonia poisoning the parasympathomimetic status predominates and could be responsible for signs such as bradycardia and profuse salivation [4]. Bradycardia was never recorded in this experiment, while profuse salivation may be caused by pharynx paralysis[2]. Additionally, both glossopharyngeal and vagal nerves that innervate the pharynx are parasympathetic and their dysfunction may cause its paralysis.

The presence of clinical signs such as horizontal nystagmus, incoordination and pushing against obstacles may reflect a dysfunction in the vestibular system and

cerebellum[16]. The former is responsible for regulating the eye muscle movement and both of them for controlling the skeletal muscle activity to maintain balance, equilibrium and coordination. Those signs may arise from lesions caused by ammonia in the brain such as neuronal degeneration and spongy degeneration of the neuropils, or due to dearrangements in the cerebral metabolism by increasing glutamate formation that depletes the Krebs cycle of a-ketoglutarate to interrupt energy metabolism and ATP synthesis[5]. Ammonia *per se* decreases postsynaptic inhibition of cortical, spinal, and trochlear motor neurons leading to severe tonic convulsions[4].

Hypothermia occurred concomitantly or son after the convulsion episodes. A low rectal temperature was detected even in cattle that exhibited more than three convulsion episodes. The higher the hematocrit, the lower the rectal temperature (r = -0.60) suggesting that hypothermia could be associated with a hypovolemic shock. Hypothermia could also reflect a reduction in the metabolic activity, which is an indicator of terminal stages in many diseases, since the steers that exhibited the lower body temperature had also flaccid musculature and nervous depression, despite the convulsion. On the other hand, some authors stated that the rectal temperature rose after convulsion in cattle and sheep, reaching its peak before death, justifying that the high body temperature was due to excessive muscular contractions.[2,17] The present results contradicted the classical descriptions since the lowest rectal temperature (35.4º C) was detected in the steer that succumbed, after five convulsive episodes.

Half of the poisoned cattle exhibited ingurgitated episcleral veins. The presence of this sign could indicate that congestion in the cerebral vessels is taking place as described elsewhere [4], because the episcleral veins are derived from the carotid artery.

Rumen stasis was seen in all poisoned steers, particularly after the appearance of muscle tremors, suggesting that high levels of ammonia could interfere with the gastric center in the hindbrain. Only one steer was bloated, probably because the animals were rumen-cannulated, while other authors recorded these signs in most poisoned cattle or sheep [2, 4]. There was significant increase in the respiratory rate in both groups (Table 3), mainly at the moments preceding the convulsion episodes, although still within the physiological range (15 – 30 mov/min) [13].

Conclusions

Although cattle fed extruded urea delayed the onset of clinical picture both prilled and extruded urea poisoned the animals with the same intensity. Besides the classical clinical signs 3 new additional signs were also recorded in the steers: dehydration, hypothermia, and ingurgitated episcleral veins. All steers that exhibited lung edema had severe dehydration. At the convulsion, animals became hypothermic, had tachycardia, rumen stasis, and increased the respiratory rate. High levels of extruded urea are as dangerous as prilled urea to cause ammonia poisoning in cattle.

Resumo

Doze garrotes Girolando, nunca alimentados com uréia, foram distribuídos em dois grupos de seis animais cada. Ambos os grupos receberam intraruminalmente dose única (0,5 g/kg PV) de uréia extrusada (G1) ou granulada (G2), para induzir quadro de intoxicação por amônia. O quadro clínico exibido pelos garrotes foi acompanhado durante 240 minutos. Além da constatação dos sinais clínicos clássicos ligados a essa intoxicação, o presente trabalho descreve a presença de três novos sinais: desidratação, hipotermia e vasos episclerais ingurgitados. Convulsão, considerada sinal

Palavras-chave:
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definitivo, ocorreu em cinco de seis animais de cada grupo. Um garrote (G1) exibiu apenas fasciculações, enquanto outro (G2) desenvolveu quadro clínico típico, porém sem convulsão, e recuperou-se espontaneamente sem tratamento. Os surgimentos de tremores musculares, decúbito esternal e episódios convulsivos ocorreram em momentos similares em ambos os grupos, mas quando analisados conjuntamente verificou-se que foram mais tardios no G1 ($p < 0,04$). O 1º sinal clínico observado foi a fasciculação, seguida por apatia, hiperestesia, apoio em obstáculos, tremores musculares, atonia ruminal, incoordenação motora, decúbito esternal e lateral, desidratação leve ou severa, e convulsão. Maiores freqüências cardíacas foram detectadas na convulsão. Após a convulsão, quatro garrotes de cada grupo apresentaram hipotermia leve. Um garrote do G2 entrou em estado comatoso e sucumbiu subitamente antes que fosse iniciado o tratamento. Apesar da uréia extrusada adiar o surgimento do quadro clínico, os sinais evidenciados foram tão severos quanto os causados por uréia granulada. Ambas formas de uréia, oferecidas em altas doses são perigosas a bovinos que nunca foram alimentados com uréia.

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