Evaluation of biochemical parameters of beef cattle suffering natural urea poisoning

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SUMMARY

Urea poisoning is one of the leading causes of accidental poisoning in cattle herds. The presented study aimed to evaluate the clinical and biochemical findings of natural urea poisoning in Angus heifers.

Clinical signs including dyspnea, ataxia, and spasms were observed in four animals from the two paddocks where the initial feed ration was distributed.Urea poisoning was suspected, serum and plasma samples were obtained from 4 sick animals(group GU) and 4 healthy animals located in different paddocks that had not yet consumed feed (group GC). Blood ammonia, albumin, Alkaline Phosphatase(ALP), Aspartate Aminotransferase(AST), Copper(Cu), -Hydroxybutyric acid(BHBA), Blood urea nitrogen(BUN), Phosphorus, Gamma-glutamyltransferase(GGT), Glutamate dehydrogenase(GLDH), Glucose, Calcium(Ca), cholesterol, Magnesium(Mg), Non-esterified fatty acids(NEFA), Total Protein(TP) and Triglyceride(TG) analyzes were performed.

Although urea had been routinely supplemented at 30 g/animal/day, investigation revealed that moisture exposure caused urea solidification in sacks, leading to inadequate homogenization within the TMR. Rumen pH was determined to be above 8.5 in all poisoned animals. A difference was determined in average ammonia (GU=456 µmol/l; GC= 272 µmol/l), AST (GU=138 U/L; GC=75.6U/L), Phosphorus (GU=2.45 mmol/L; GC=1.83 mmol/L), BUN (GU=10.71 mmol/L; GC= 9.28 mmol/L) and Triglyceride (GU=0.51 mmol/L; GC=0.17 mmol/L) levels in the GU and GC groups.

Routine parameters including AST, phosphorus, triglycerides, and BUN - unlike ammonia which requires specific handling - may serve as practical diagnostic markers for urea poisoning in field conditions.

KEY WORDS

Ammonia; AST; Phosphorus; Triglyceride; BUN.

INTRODUCTION

Urea is an organic compound used as a non-protein nitrogen (NPN) protein source in cattle rations. Urea is taken into the rumen and is converted into free ammonia, which is used as a nitrogen source for the rumen flora, thereby increasing microbial protein synthesis. Since 100 grams of urea as a nitrogen source is equivalent to approximately 287 grams of microbial protein, urea feeding is considered an economical practice in terms of animal nutrition[1]. The narrow margin between metabolic utilization and toxic thresholds makes urea-fed ruminants susceptible to poisoning. Accordingly, when urea is added to the ration, it is recommended that its amount should not exceed 1% of the concentrated feed, 20% of the protein when other NPN sources are taken into consideration, and 135 grams per animal per day[2].

Poisoning may occur if animals are given high amounts of urea, if the urea is not homogenized in the ration, and if the animals are suddenly fed too much urea without adaptation[3-5]. In case of poisoning, the urea taken with the diet is converted into ammonia by the urease enzyme in the rumen. Still, since it cannot be used entirely by the rumen microflora, it is absorbed by the rumen wall and transported to the liver by the portal circulation. In the liver, ammonia is converted into urea, 25-60% of which is eliminated with urine, and some of it is included in urea recycling through saliva[6]. However, in cases where the amount of ammonia is very high, hepatocytes are insufficient to ensure this conversion, and the ammonia level in the blood increases. Ammonia blocks the Krebs cycle by binding the glutamine-synthetase enzyme within the cell, thus disrupting cellular respiration[1]. When the level of ammonia in the blood increases, ammonia can cross the blood-brain barrier and has a neurotoxic effect by disrupting the synthesis of histamine, serotonin, dopamine and noradrenaline, causing the enlargement of astrocytes, disrupting synaptic transmission and causing different molecules to cross the blood-brain barrier[7]. As a result, a clinical presentation of urea poisoning characterized by nervous symptoms occurs within minutes to first hours following incorrect use of urea in animals.

Symptoms in urea poisoning are usually peracute and the fatality rate is high. Clinical findings observed shortly after urea ingestion include excitement, apathy, hypersalivation, incoordination, rapid and difficult breathing, moaning, tremors that start from the head and neck and then become generalized, and subsequent coma/death[8,9]. The fact that the disease progresses very quickly and therefore animals are often found dead, and

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blood ammonia measurement is required for definitive diagnosis, may cause urea poisoning cases not to be diagnosed correctly and, accordingly, the magnitude of the problem to be not clearly seen.

The presented study aimed to present a case in a farm where natural urea poisoning occurred and to compare the blood ammonia and some other biochemical parameters of poisoned and healthy animals.

MATERIAL AND METHOD

The study was approved by Bursa Uludag University local ethichs comitee (No: 2024-05/01) The study was conducted in December in a farm located in the south of Turkey. The farm imported 8000 Angus heifers a month ago. Starting from the third day of their arrival at the farm, the animals were adapted to urea feeding by increasing the amount and fed with the ration in Table 1.

Within 45 minutes following feeding, incoordination, apathy, hypersalivation, rapid and difficult breathing, and generalized tetany were observed in 4 animals in the two paddocks where feeding was first done. Following the detection of clinical signs, feeding was stopped, the mixer was emptied and a urea-free ration was prepared for the remaining animals. One of the four sick animals was given 2 liters of vinegar mixed with 2 liters of warm water. Sample collection

In the study, 4 animals experiencing natural urea poisoning constituted the study group (GU). Four pregnant heifers of the same breed and age, selected from paddocks that had not yet shed TMR, were randomly selected to form the control group (GC). After measuring body temperature and respiratory rate of all animals in the GU group, rumen content and a urine sample of an animal that urinated at this time were taken. Rumen contents and pHs from the urine sample were measured using Test Paper Strip (0-14,DF, Guangzhou China).

Subsequently, blood was collected from the animals in the GU and GC groups from the jugular vein into anticoagulant tubes containing EDTA and also to plain tubes, serum and plasma samples were obtained by centrifuging at 1008 g for 15 minutes. The samples obtained were frozen at -20 °C and then sent to the laboratory where analyzes were performed in the cold chain. The next day, serum samples were analyzed for albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), copper, beta-hydroxybutyric acid (BHBA), blood urea nitrogen (BUN), phosphorus, gamma glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), glucose, calcium, cholesterol, magnesium, non-esterified fatty acids (NEFA), total protein and triglyceride, using an automatic autoanalyzer (Prestige 24i Tokyo-Boeki, Japan). Ammonia analysis was performed on a fully automatic autoanalyzer (Prestige 24i Tokyo-Boeki, Japan) using a commercial kit (COD 23532, BioSystems S.A., Spain). Ammonia analysis could be performed 4 days after the samples were taken due to the time required for the kit to be supplied by the laboratory.

Statistical analyses were performed using Sigmaplot software (Version 15; Systat software CA, USA). Data were examined for normality distribution (Shapiro-wilk test) and variance homogeneity assumptions (Brown-Forsythe test). If normally distributed, t test was applied, and the differences between groups were analyzed. The differences were considered significant at $P<0.05$.

Table 1 - Ration fed to study animals.

Feed	Quantity (kg)/Heifer
Corn silage	10
Ryegrass hay	0.5
Maize stover	0.75
Wheat hay	1
Corn	0.4
Barley	0.35
Cottonseed meal	1
Wheat bran	0.5
Urea	0.03
Buffer premix	0.2

RESULTS

All four affected animals succumbed within 90 minutes of clinical onset.In the general examination of the animals in the GU group, the body temperature of 3 animals was normal. In contrast, the body temperature of one heifer was 39.9 °C. The average respiratory frequency of animals in the GU group was 76/min (min.:=68/min; max.=88/min). Rumen pH values were determined to be between 8-8.5 in 4 animals suffering from urea poisoning. In an animal whose urine pH was measured, the urine pH was measured as 8. In the examination carried out in the feed store, it was determined that the urea fed to the animals got wet and became lumpy. The biochemical analysis results determined that ammonia, triglyceride, phosphorus, BUN and AST concentrations were significantly higher in the GU group than in the GC group (Table 2). On the other hand, no difference was determined between the groups in the other evaluated parameters, namely serum total protein, NEFA, magnesium, cholesterol, calcium, glucose, GLDH, GGT, Copper, ALP and albumin concentrations.

DISCUSSION

Due to its low cost, urea is widely used in cattle nutrition as a source of N. Due to this intensive use, herds have a high risk of accidental urea poisoning. In a survey conducted with 35 veterinarians in Brazil, although 88.9% of the physicians reported encountering clinical symptoms similar to urea poisoning at least once, 87.5% stated that they did not perform necropsy or send samples to the laboratory to confirm the diagnosis[1]. The main reason for this is that the definitive diagnosis of urea poisoning is based on blood and rumen ammonia levels[10]. Accordingly, rumen ammonia level over 1000 mg/l and blood ammonia level over 20 mg/l were determined as indicators of urea poisoning. In the presented study, although plasma ammonia concentration was significantly higher in the poisoning group than in the control group (456.1µmol/l = 7.76 mg/dl), it was determined below the 20 mg/l level specified in the above study. In a study where ammonia levels similar to the presented study were determined, it was reported that clinical symptoms started when plasma ammonia levels were at 782 µmol/l in steers

Table 2 - Biochemical analysis results of poisoned heifers in GU and control heifers in GC groups.

	GROUPS		
	GU(study grup)	GC(control grup)	*P*
Ammonia (µmol/l)	456.1 ± 12.1	271.5 ± 11.8	<0.001
Triglyceride (mmol/L)	0.51 ± 0.01	0.17 ± 0.002	<0.001
Total Protein (g/L)	66.2 ± 4.0	62.0 ± 1.2	n.s.
NEFA (1 mEq/L)	0.45 ± 0.05	0.59 ± 0.09	n.s.
Magnesium (mmol/L)	0.71 ± 0.004	0.71 ± 0.008	n.s.
Cholesterol (mmol/L)	2.09 ± 0.36	2.59 ± 0.17	n.s.
Calcium (mmol/L)	2.16 ± 0.015	2.16 ± 0.015	n.s.
Glucose (mmol/L)	3.24 ± 0.036	3.08 ± 0.078	n.s.
GLDH (U/L)	16.2 ± 0.6	18.0 ± 0.6	n.s.
GGT (U/L)	10.8 ± 0.6	12.6 ± 1.8	n.s.
Phosphorus (mmol/L)	2.45 ± 0.026	1.83 ± 0.10	<0.001
BUN (mmol/L)	10.71 ± 0.39	9.28 ± 0.14	<0.05
Copper (IU/l)	93.42 ± 1.39	93.39 ± 1.05	n.s.
AST (U/L)	138.0 ± 0.6	75.6 ± 4.8	<0.001
ALP (U/L)	162.6 ± 8.4	180.0 ± 2.4	n.s.
Albumin (g/l)	38.2 ± 1.8	37.2 ± 0.2	n.s.

NEFA: non-esterified fatty acids; GLDH: glutamate dehydrogenase; GGT: gamma-glutamyl transferase; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALP: Alkaline Phosphatase; n.s.: not significant

experimentally poisoned with urea[11]. This difference may be related to ammonia measurement errors or the interval of several days between obtaining the plasmas and performing the analyses. Because blood ammonia levels are not stable and ammonia levels may vary depending on many factors such as the anticoagulant in the blood tube taken, the time between collection and plasma separation process, centrifuge speed and accordingly the number of platelets remaining in the plasma, and even the brand of the autoanalyzer used for analysis[12,13].

Serum BUN concentration was determined to be higher in the poisoning group than in the control group, although the statistical difference between the groups was not as significant as determined in plasma ammonia levels. This is because ammonia has a limited capacity to convert to urea in the liver, and therefore serum urea concentrations do not show a rapid peak like ammonia. Huntington et al. stated in their study that the blood ammonia level of steers fed with a slow-release urea source peaked at the 120th minute, but serum urea concentrations continued to increase steadily[14]. In the presented study, blood samples were collected approximately 90 minutes after the animals consumed the ration that caused the poisoning, so it can be concluded that while the ammonia levels were approaching the peak, the serum urea level had not yet fully increased.

One of the main clinical findings of urea poisoning is ataxia and tetany in animals. The tetanies that occur cause muscle damage in poisoned animals. A study stated that urea poisoning caused muscle damage in steers, characterized by increased AST and CK enzyme activities, but did not cause liver damage[11]. Similar findings were determined in the presented study. Although AST was determined to be high in the group experiencing urea poisoning, the fact that ALP and GLDH enzyme activities did not differ between the groups raises the possibility that the increase in AST is related to muscle damage. GLDH is an enzyme required for the de novo synthesis of glutamate required for the detoxification of ammonia, whereas hepatocytes must be damaged in order to increase the serum concentration of GLDH, as it is a mitochondrial enzyme[15]. Another finding that may be compatible with tetany, trauma and muscle damage is that serum phosphorus levels in the poisoned group were measured higher than in the control group. It has been reported that muscle damage following severe tetanic convulsions may cause hyperphosphatemia, and hyperphosphatemia becomes more pronounced in the case of myoglobinuria[16].

Blood ammonia and liver ureagenesis activity is closely related to triglyceride. In a study using bovine hepatocyte cell culture, it was determined that triglyceride accumulation significantly reduced ureagenesis capacity of the cells[17]. Similarly, a study conducted in Holstein cows determined a positive correlation between liver triglyceride accumulation and plasma ammonia concentrations[18]. In another study investigating the effect of urea supplementation on plasma metabolites in lambs, it was reported that plasma triglyceride concentration increased significantly after 4 weeks in lambs supplemented with urea[19]. In our study, serum triglyceride concentration in the poisoned group was determined to be significantly higher than in the control group. This difference may be directly related to the poisoning, or it may be related to a condition prior to the poisoning that would predispose the animals to urea poisoning. Because the symptoms of poisoning are seen only in certain animals in the paddock that feed containing urea has been used, it may be related to the clumping that occurs in the urea, or it may be associated with the pre-existing fatty liver in the animals.

In the presented study, the clinical findings observed in animals poisoned with urea and the increase in rumen pH are compatible with the literatüre[1]. After animals consume urea, it is hydrolyzed by urease synthesized by microorganisms in the ru-

men flora. As a result of hydrolysis, an increase in rumen pH occurs, which is one of the typical findings of urea poisoning[20]. Increased rumen pH is an important clinical finding and also plays an important role in the pathogenesis of the disease. When the rumen pH is in the range of 6-7, most of the ammonia absorbed from the rumen wall is NH_4^+. On the other hand, if the rumen pH increases, the absorbed ammonia is in the form of NH_3, and the increased pH causes the absorption of ammonia from the rumen wall to increase and intensify the toxicity[21]. The reason for using vinegar or acetic acid in the treatment of urea poisoning is to reduce ammonia absorption by lowering the rumen pH. In the presented study, although a heifer was treated with vinegar, the patient could not be saved. This may be related to the amount of urea consumed by the animal and the time of intervention.

As a result, urea is used in cattle nutrition as a cost-effective protein source. Although clinical findings and rumen pH are useful in the diagnosis of poisoning, plasma ammonia concentrations must be determined for a definitive diagnosis. However, ammonia measurement is not practical because ammonia analyzes are not routine and many factors can cause errors in the results. Although the number of animals in the presented study was low, the change in biochemical parameters that can be evaluated routinely can support the clinical diagnosis of poisoning.

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Author Contributions

Zafer Mecito lu: Conceived and designed the study. Analyzed the serum samples. Analyzed and evaluated the data. Wrote the manuscript.

Mehmet Emin Akkaş: Applied the experiment and analyzed the serum samples. Analyzed the data.

Conflict of Interest Statement

The authors declare no conflict of interest.

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