

INFLUENCE OF TOXIC DOSES OF NITRATE AND SACCHAROSE UPON SOME BLOOD, URINE AND RUMEN BIOCHEMICAL INDICES IN SHEEP

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Summary

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The experiments were performed on 12 sheep divided into two groups: group I, treated orally with 0.65 g/kg b.w. potassium nitrate and 10.0 g/kg b.w. saccharose dissolved in 1.5 l water and group II, treated similarly with 1.0 g/kg b.w. potassium nitrate and 20.0 g/kg b.w. saccharose two hours after the morning feeding. At hours 2, 4, 6, 8, 10, 12, 24 and 48, samples of blood, urine and rumen liquid were obtained for determination of pH and the concentrations of nitrates and nitrites.

It was found out that rumen pH decreased after the 6th hour, being significantly ($p<0.05$) lower at hour 10 and 12 (5.6 ± 0.2 for group I and 5.00 ± 0.04 for group II, respectively) compared to hour 0. Blood pH decreased after the 4th hour in both groups and reached the minimum values at hour 24 (7.15–7.16). Urine pH also decreased to 6.30 ± 0.20 (post treatment hour 12) and 6.70 ± 0.20 (post treatment hour 24) at $p<0.05$.

The concentration of rumen nitrates was elevated at hour 2 and it persisted up to hour 24. Rumen nitrites were also increased with peak values by hours 8–10. The amounts of nitrates and nitrites in blood and urine were increased proportionate to increased rumen concentrations. The lower rumen pH delayed or stopped the reduction of nitrates in rumen. The simultaneous ingestion of toxic doses of nitrates and saccharose in ruminants (sheep) was less poisonous compared to their independent administration.

Key words: blood, intoxication, nitrates, nitrites, pH, rumen liquid, saccharose, sheep, urine

INTRODUCTION

Nitrate intoxication and rumen acidosis in sheep, occurring separately, have been subject to multiple studies (Dirksen, 1970; Huber, 1976; Popov et al., 1982; Popova et al., 1982; Nicolov, 1984; Malchevski, 1985; Crichlow, 1989; Goranov et al., 1990; Ganter et al., 1993; Nicolov & Kunev, 1993b; Yoon & Kim, 1993; Nikolov & Groseva, 1993; Nikolov, 1996). Thus, there is a lot of information for their toxicodynamics, pathogenesis, diagnostics

etc. The dominating sign in acute nitrate poisoning is methaemoglobinaemia, that appears after hour 4 of the intoxication and is responsible for the lethal issue in many animal species (Nikolov, 1984; Boermans, 1990; Yoon and Kim, 1993). The ingestion excessive amounts of easily digested carbohydrates resulted in severe metabolic disorders in ruminants, manifested by hyperglycaemia, lactacidaemia etc. (Dirksen, 1970; Vihan, 1978;

Randhawa et al., 1980; Das and Misra, 1991; Lal et al., 1991; Lal et al., 1992; Ganter et al., 1993; Sen et al., 1993; Nikolov, 1996). Data about intoxications, due to the simultaneous ingestion of toxic doses of nitrates and easily digested carbohydrates are however less numerous.

In previous studies of ours (Nikolov, 2000; Nikolov, 2001) upon the clinical, haematological and some biochemical blood parameters in sheep, having simultaneously ingested toxic doses of nitrates and saccharose, we reported changes that differed chronologically compared to those due to independent administration of excessive amounts of nitrates and easily digested carbohydrates. In the available literature, there were no data about the changes in some biochemical parameters of blood, rumen liquid and urine from ruminants that received toxic doses of nitrates and carbohydrates at the same time. This fact motivated our study, that aimed to evaluate the effect of toxic doses of these substances on some biochemical parameters of blood, rumen liquid and urine in sheep.

MATERIALS AND METHODS

The experimental studies were performed on 12 clinically healthy Merino sheep weighing 52-62 kg, aged 5-6 years. All animals were housed in appropriate conditions. Prior to the experiments, the sheep were treated against endo- and ectoparasites *s.c.* with 200 µg/kg ivermectin (Ivomec, Merck Sharp & Dohme Ltd.). The daily ration included 0.5 kg concentrate, 1 kg alfalfa hay, 4 kg corn silage per animal with free access to water and salt.

Blood (*v. jugularis*), urine and rumen liquid samples were obtained in four consecutive days prior to the treatment (hours -72, -48, -24 and 0) 2 hours after the morning feeding for spectrophotometrical

determination of nitrate and nitrite concentrations (Ibrishimov and Lalov, 1984). Blood pH values were determined by a blood gas analyser (ABL-3, Radiometer, Denmark) while rumen and urine pH - by a pH-meter (AP-17, Hungary). Those data served as controls.

Prior to the experimental intoxication, the sheep were divided into two experimental groups, six animals each (group I and II). The sheep from group I were treated at hour 0 with potassium nitrate at a dose of 0.65 g/kg b.w. (corresponding to 1/3 of the oral LD₅₀ for rabbits) and saccharose at a dose of 10.0 g/kg b.w. (about 10 times higher than the therapeutic one) dissolved in 1500 ml water. The sheep from experimental group II were identically treated with 1.0 g/kg b.w. potassium nitrate and 20.0 g/kg b.w. saccharose. Both substances were applied 2 hours after the morning feeding via a stomach tube.

All forementioned parameters were assessed at hours 2, 4, 6, 8, 10, 12, 24 and 48 hours after the treatment. The statistical significance of results was determined using the Student t-test at a level of significance of $p < 0.05$ vs zero values.

RESULTS

The data obtained in the period prior to the study, showed values with small fluctuations for all studied parameters.

The changes in rumen pH are presented in Table 1. It was found out that prior to the treatment with potassium nitrate and saccharose, pH was within the physiological limits: 6.9 ± 0.2 – 7.1 ± 0.2 for group I and 6.8 ± 0.3 – 7.1 ± 0.1 for group II. During the development of the acute combined nitrate and carbohydrate intoxication, it decreased and by hour 6, pH values for both groups were 6.6 ± 0.2 and 5.8 ± 0.1 respectively. By hours 10–12

Table 1. Changes in rumen liquid, blood and urine pH in sheep, simultaneously treated orally with 0.65 g/kg b.w. potassium nitrate and 10.0 g/kg b.w. saccharose (group I, n=6) or with 1 g/kg b.w. potassium nitrate and 20.0 g/kg b.w. saccharose (group II, n=6). The values are presented as mean $\pm$ SEM

Samples	Group	Hours prior to treatment						
		-72	-48	-24	0			
Rumen liquid	I	7.1 $\pm$ 0.2	6.9 $\pm$ 0.4	7.1 $\pm$ 0.2	6.9 $\pm$ 0.2			
	II	7.0 $\pm$ 0.3	7.1 $\pm$ 0.1	6.8 $\pm$ 0.3	7.1 $\pm$ 0.1			
Blood	I	7.40 $\pm$ 0.01	7.41 $\pm$ 0.02	7.42 $\pm$ 0.01	7.40 $\pm$ 0.02			
	II	7.41 $\pm$ 0.02	7.40 $\pm$ 0.01	7.40 $\pm$ 0.02	7.41 $\pm$ 0.03			
Urine	I	7.95 $\pm$ 0.02	7.94 $\pm$ 0.03	7.91 $\pm$ 0.2	7.93 $\pm$ 0.03			
	II	7.83 $\pm$ 0.03	7.88 $\pm$ 0.04	7.94 $\pm$ 0.2	7.92 $\pm$ 0.04			
Hours post treatment								
Rumen liquid	I	7.0 $\pm$ 0.2	6.8 $\pm$ 0.2	6.6 $\pm$ 0.2	6.5 $\pm$ 0.1	6.1 $\pm$ 0.2 ^a	5.6 $\pm$ 0.2 ^a	6.1 $\pm$ 0.1 ^a
	II	6.9 $\pm$ 0.1	6.3 $\pm$ 0.3	5.8 $\pm$ 0.1 ^a	5.1 $\pm$ 0.3 ^a	5.0 $\pm$ 0.3 ^a	5.0 $\pm$ 0.4 ^a	5.8 $\pm$ 0.1 ^a
Blood	I	7.39 $\pm$ 0.03	7.33 $\pm$ 0.02 ^a	7.31 $\pm$ 0.02 ^a	7.28 $\pm$ 0.01 ^a	7.2 $\pm$ 0.02 ^a	7.20 $\pm$ 0.02 ^a	7.16 $\pm$ 0.01 ^a
	II	7.39 $\pm$ 0.02	7.30 $\pm$ 0.03 ^a	7.29 $\pm$ 0.02 ^a	7.25 $\pm$ 0.01 ^a	7.18 $\pm$ 0.03 ^a	7.17 $\pm$ 0.02 ^a	7.15 $\pm$ 0.01 ^a
Urine	I	7.89 $\pm$ 0.02	7.22 $\pm$ 0.02	7.11 $\pm$ 0.01 ^a	6.8 $\pm$ 0.2 ^a	6.7 $\pm$ 0.2 ^a	6.7 $\pm$ 0.1 ^a	7.3 $\pm$ 0.1
	II	7.7 $\pm$ 0.3	7.5 $\pm$ 0.1	7.21 $\pm$ 0.02 ^a	6.7 $\pm$ 0.3 ^a	6.6 $\pm$ 0.1 ^a	6.3 $\pm$ 0.2 ^a	6.7 $\pm$ 0.2 ^a

^a p<0.05 vs values at hour 0.

were observed the lowest values (5.6 ± 0.2 (group I) and 5.0 ± 0.4 (group II) ($p < 0.05$). Afterwards, pH increased and by hour 48 reached the initial levels. Rumen pH changes were accompanied by changes in blood and urine pH as well. The analysis of blood gases revealed that blood pH was significantly lower than control determinations by post treatment hour 4 (7.33 ± 0.02 for group I and 7.30 ± 0.03 for group II, $p < 0.05$) (Table 1). By hours 6, 8, 10, 12 and 24, pH progressively decreased (7.20 ± 0.02 for group I and 7.17 ± 0.02 for group II at hour 12, $p < 0.05$) and then began to augment. Similar changes were recorded for urine pH (Table 1). Being 7.91 ± 0.20 – 7.95 ± 0.02 and 7.83 ± 0.03 – 7.94 ± 0.20 respectively prior to the treatment, they decreased from hour 6th onward and reached the lowest values by hours 10–12: 6.7 ± 0.1 and 6.3 ± 0.2 for groups I and II, respectively ($p < 0.05$).

The alterations in rumen nitrate and nitrite concentrations prior to and after the treatment of sheep with potassium nitrate and saccharose are presented in Table 2. The control determinations (from hour -72 to hour 0) gave values between 150 ± 6.1 ppm and 161 ± 6.7 ppm for nitrates and 0.9 ± 0.01 ppm and 1.4 ± 0.2 ppm for nitrites.

After the administration of the toxic doses of both substances, a sharp increase followed. By hour 2, the levels of nitrates were 655 ± 10.1 ppm for group I ($p < 0.001$) and 720 ± 11.1 ppm for group II ($p < 0.001$). These high levels persisted also at post treatment hour 6 (580 ± 12.1 ppm and 685 ± 13.2 ppm respectively). Between hours 8 and 12, they were 495 ± 9.5 – 610 ± 8.2 ppm, then decreased and by hour 48 reached the initial values.

Rumen nitrite levels showed a tendency towards significant increase compared to baseline determinations as early

as hour 2: 2.3 ± 0.2 ppm ($p < 0.05$) in group I and 2.6 ± 0.3 ppm ($p < 0.05$) in group II. Peak values were measured between hours 4 and 8, followed by reduction up to hour 48.

Blood concentrations of nitrates and nitrites (Table 2) in all sheep prior to the treatment were within the physiological ranges (10.2 ± 0.3 – 11.1 ± 0.4 ppm for nitrates and 0.5 ± 0.01 – 0.8 ± 0.02 ppm for nitrites). After the combined administration of nitrate and saccharose, the amount of nitrates increased in the early hours (hour 2) to 28.3 ± 1.1 ppm and 35.4 ± 1.6 ppm ($p < 0.05$) for groups I and II respectively. The progressive elevation was observed up to hour 24, when the peak values were displayed: 110.5 ± 9.1 ppm (group I) and 130.1 ± 6.9 ppm (group II) ($p < 0.001$). By hour 48 the concentrations decreased but did not reach the initial values.

Blood nitrite levels increased in the first post treatment hours being the maximum by hour 8 in group I – 2.7 ± 0.2 ppm and in group II – 2.9 ± 0.3 ppm ($p < 0.001$). After the 12th hour and up to the end of the experimental period, their values varied closely to initial ones.

The amounts of nitrates in the urine of sheep prior to the treatment were between 12.3 ± 1.2 ppm and 15.3 ± 1.3 ppm and those of nitrites – 0.8 ± 0.01 and 1.1 ± 0.08 ppm. After the intoxication, the amounts of nitrates, excreted with urine increased to peak values by hours 12–24 (160.0 ± 6.2 – 180.0 ± 8.1 ppm for group I and 170.0 ± 5.9 – 200.0 ± 9.2 ppm for group II, $p < 0.001$).

A statistically significant elevation of urine nitrites in both groups was observed by post treatment hours 6–8 (being the maximum by hour 6 – 1.5 ± 0.21 ppm and 1.7 ± 0.3 ppm respectively, $p < 0.05$).

Table 2. Changes in rumen liquid, blood and urine nitrate and nitrite concentrations (ppm) in sheep, simultaneously treated orally with 0.65 g/kg b.w. potassium nitrate and 10.0 g/kg b.w. saccharose (group I, n=6) or with 1 g/kg b.w. potassium nitrate and 20.0 g/kg b.w. saccharose (group II, n=6). The values are presented as mean $\pm$ SEM

Samples	Substances	Group	Hours prior to treatment							
			-72	-48	-24	0				
Rumen liquid	Nitrates	I	150.0±6.1	161.0±6.7	158.0±8.4	154±10.2				
		II	153.0±7.2	156.0±9.3	160.0±10.1	158±7.5				
	Nitrites	I	1.10±0.09	0.90±0.01	1.2±0.1	1.3±0.3				
		II	1.20±0.08	1.30±0.07	1.0±0.2	1.4±0.2				
Blood	Nitrates	I	10.2±0.3	10.5±0.6	11.0±1.1	10.8±0.9				
		II	11.1±0.4	10.9±0.9	11.0±0.9	10.7±1.5				
	Nitrites	I	0.50±0.01	0.60±0.01	0.50±0.02	0.7±0.1				
		II	0.70±0.02	0.50±0.02	0.70±0.03	0.8±0.02				
Urine	Nitrates	I	12.3±1.2	13.4±0.9	12.5±0.7	13.2±0.9				
		II	13.1±0.8	14.2±1.5	15.3±1.3	14.1±1.1				
	Nitrites	I	0.80±0.01	1.0±0.2	1.10±0.07	0.90±0.01				
		II	0.90±0.1	1.10±0.08	0.80±0.08	0.80±0.03				
			Hours post treatment							
Rumen liquid	Nitrates	I	655±10.1 ^c	650±11.2 ^c	580±12.1 ^c	500±8.6 ^c	509±8.1 ^c	495±9.5 ^c	208±14.1 ^c	48
		II	720±11.1 ^c	710±15.6 ^c	685±13.2 ^c	600±9.3 ^c	602±10.1 ^c	610±8.2 ^c	310±10.1 ^b	186±9.2
	Nitrites	I	2.3±0.2 ^a	5.5±0.3 ^c	5.6±0.2 ^c	5.0±0.4 ^c	4.9±0.2 ^c	4.3±0.1 ^b	1.5±0.1	193±8.3
		II	2.6±0.3 ^a	6.4±0.5 ^c	6.2±0.4 ^c	6.3±0.2 ^c	6.0±0.3 ^c	5.1±0.3 ^c	2.2±0.1	0.9±0.1
Blood	Nitrates	I	28.3±1.1 ^a	39.4±3.2 ^b	50.1±5.2 ^c	61.3±4.1 ^c	79.8±6.2 ^c	93.5±7.3 ^c	110.5±9.1 ^c	1.5±0.2
		II	35.4±1.6 ^a	48.1±3.8 ^b	60.1±6.1 ^c	75.4±5.9 ^c	98.3±7.3 ^c	119.4±9.1 ^c	130.1±6.9 ^c	34.5±3.5
	Nitrites	I	0.90±0.01	1.3±0.1 ^b	1.8±0.3 ^b	2.7±0.2 ^c	1.5±0.3 ^b	0.60±0.09	0.20±0.01	49.6±3.5
		II	1.0±0.09	1.80±0.09 ^b	2.2±0.4 ^c	2.9±0.3 ^c	1.8±0.4 ^b	0.50±0.09	0.40±0.02	0.30±0.01
Urine	Nitrates	I	40.1±4.2 ^a	55.3±4.9 ^a	78.5±6.5 ^c	95.4±8.9 ^c	110.0±9.5 ^c	160±6.2 ^c	180±8.1 ^c	0.60±0.02
		II	50.5±3.9 ^a	60.2±5.3 ^b	88.3±7.8 ^c	110±9.1 ^c	152.3±10.1 ^c	170±5.9 ^c	200±9.2 ^c	54.1±4.3
	Nitrites	I	0.80±0.01	0.90±0.01	1.50±0.21 ^a	1.3±0.2 ^a	1.1±0.2	0.9±0.1	0.70±0.04	48.3±3.1
		II	0.90±0.02	1.2±0.1	1.7±0.3 ^a	1.6±0.1 ^a	1.2±0.3	0.80±0.03	0.90±0.02	1.0±0.1
			1.2±0.3						0.90±0.02	1.1±0.2

^a p<0.05; ^b p<0.01; ^c p<0.001 vs values at hour 0.

^a p<0.05; ^b p<0.01; ^c p<0.001 vs values at hour 0.

DISCUSSION

The studied parameters in rumen liquid, blood and urine in sheep treated concurrently with toxic doses of nitrates and saccharose were indicative for severe systemic metabolic disorders. Both substances, simultaneously administered (in doses that provoke toxic manifestations during independent application in sheep), undergo a degradation in rumen via independent biochemical pathways up to hour 2, without any interference (Nikolov, 2000; Nikolov, 2001). The saccharose, ingested orally in toxic doses (10 and 20 g/kg b.w.) is undergoing microbial fermentation by rumen microflora. The end products of that fermentation are ethanol, carbon dioxide, volatile fatty acids, lactic acid etc. The lactic acid as well as carbon dioxide are formed in the greatest amounts while the volatile fatty acids change their percentage in favour of propionic and butyric acids with decrease of acetic acid (Dirksen, 1970; Malchevski, 1985; Crichlow, 1989; Nikolov & Kunev, 1993b; Angelov et al., 1994; Nikolov, 1996). At the same time, a liberation of histamine also occurs. This impaired balance of acids and biogenic amines in rumen results in decrease in rumen pH after the 4th hour, particularly in sheep from group II, treated with 20 g/kg b.w. saccharose. The lowest pH values were attained between post treatment hours 8 and 12 (reaching 5.0 ± 0.3). The decreased rumen pH creates a prerequisite for the moving of liquids from blood to rumen because of its higher osmotic pressure. The volume of rumen is increased and its content is liquid, aqueous and with an sour odour. The organism of the animal is dehydrated (Nielson, 1974; Crichlow, 1989; Brunning and Kaneene, 1993; Nikolov, 2000; Nikolov, 2001).

The formed lactic acid and the other

metabolites of carbohydrate fermentation in rumen are absorbed in blood and influence blood acid-base balance. Our results showed that up to hour 2 after the treatment of sheep with saccharose and potassium nitrate, blood pH was within the reference range as result of the compensated metabolic acidosis. Afterwards (hours 6–24) it became decompensated and blood pH was significantly under the normal values. Lactacidaemia influences urine pH in both groups, that was lower between hours 6 and 24. Similar results were reported by other authors following independent administration of saccharose (Angelov et al., 1994).

Nitrates, ingested with forages (or during an experimental study) are reduced in the forestomachs of ruminants to nitrites, hydroxylamine and ammonia. This reduction is performed by nitrate reductase producing bacteria (Alexander, 1972; Berschneider & Hornicke, 1979; Korzeniowski & Geurink, 1980; Nikolov, 1984; Boermans, 1990; Nikolov, 1984). When the ration does not contain easily digestible carbohydrates, the reduction of nitrates to nitrites by microbial nitrate reductases occurs faster and in a greater amount than in the presence of such substances (Buck & Osweiler, 1976; Malestein & Geurink, 1980; Samol & Socolowski, 1980). Our studies suggested that at hour 2 after the treatment of sheep with potassium nitrate and saccharose, rumen nitrate concentrations in both groups was 4–5 times higher than initial values and these concentrations persisted up to hour 6. The conversion of nitrates to nitrites continued up to hour 8 when rumen pH was measured to be 5.1 This is in agreement with data, that the reduction of nitrates to nitrites is decreased and more difficult when pH is lower than 6.0, and stops when it falls under 5.0. The sharp

decrease in rumen pH impairs the production of microbial nitrate reductase (Alexander, 1972; Buck & Osweiler, 1976; Nikolov, 1984; Antoine, 1993; Boermans, 1990; Nikolov, 2000).

The reduction of nitrates in the forestomachs of ruminants via the intermediate systems (nitrate–nitrite–hydroxylamine–ammonia) by the unaffected microflora is done relatively quickly and without absorption of intermediate metabolites in blood (Waang & Rivera, 1961; Huber, 1976; Korzeniowsky & Geurink, 1980). When the rate of conversion of nitrates into nitrites exceeds the rate of conversion of nitrites into hydroxylamine and ammonia, signs of nitrate intoxication occur (Alexander, 1972; Antoine, 1993; Yoon & Kim, 1993).

The amounts of non-reduced nitrate in rumen remain unchanged, one part of them being evacuated to the other forestomachs and excreted by faeces while another part is absorbed in blood and blood levels are respectively increased. The nitrates in the circulation are excreted via the kidneys with urine, where their concentrations were shown to be elevated. The toxicokinetics of rumen nitrites that reach the blood circulation for a part of them is connected with oxidizing of haemoglobin to methaemoglobin (Nikolov, 2001). Another part is excreted with urine.

The pH of nitrates and nitrites is neutral to alkaline, so they neutralize part of the acid metabolites from the fermentation of saccharose in the rumen of sheep. That is why rumen pH in experimental animals decreased slowly and reached values about 5.0 after hours 8–12. Following the independent application of the same doses of saccharose in sheep, this decrease occurs between post treatment hours 4 and 12 (Vihan, 1978; Lal et al., 1991; Lal et al., 1992; Nikolov & Kunev, 1993a,

1993b; Nikolov, 1996).

Both nitrates and nitrites do not influence the microflora, fermenting the easily digestible carbohydrates in rumen, and that is why the intermediate and end metabolites of this fermentation exert their systemic toxic effect (Waang & Rivera, 1961; Malestein & Geurink, 1980; Nikolov, 1984; James et al., 1985; Rao et al., 1995).

CONCLUSIONS

In our studies on sheep aiming to follow out the effect of toxic doses of nitrates and easily digestible carbohydrates (saccharose) we observed that the primary effect was that of carbohydrate fermentation metabolites. They influenced negatively the chain rumen–blood–urine, decreasing their pH values during various periods of the experiment. The change in rumen pH slowed down (up to hour 8) and stopped (between hours 8–12) the nitrate reduction in rumen thus contributing to the lack of clinical manifestations of nitrite intoxication although the ingestion of toxic doses of potassium nitrate. The simultaneous ingestion of excessive doses of potassium nitrate and saccharose by sheep was less poisonous compared to their independent administration.

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