

INFLUENCE OF TOXIC DOSES OF NITRATE AND SACCHAROSE UPON SOME HAEMATOLOGICAL AND BLOOD BIOCHEMICAL PARAMETERS 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 72, 48, 24 prior to the treatment and immediately before it - hour 0 blood samples were obtained from v. jugularis for determination of haemoglobin and methaemoglobin concentrations, red blood cells (RBC) and white blood cells (WBC) counts, the erythrocyte sedimentation rate (ESR), the differential WBC counts and the amounts of blood sugar and lactate. Those parameters were followed out after the treatment of both groups at hours 2, 4, 6, 8, 10, 24 and 48.

It was determined that haemoglobin concentrations decreased following post treatment hour 4. They ranged between 6.2-6.3 g/100 ml for group I and 5.7 – 5.9 g/100 ml for group II ($p<0.05$). Methaemoglobin concentrations were increased up to hour 6 to 9.0 ± 0.3 % of total haemoglobin (group I) and 18.1 ± 1.2 % (group II, $p<0.05$). A clear erythrocytosis, leukocytosis, delayed ESR, lactacidaemia and hyperglycaemia (after hour 6) were observed. Those haematological and biochemical parameters could be used as markers in the diagnostics of this pathology condition in sheep.

Key words: blood sugar, blood lactate, erythrocytes, leukocytes, haemoglobin, methaemoglobin, intoxication, nitrates, saccharose, sheep

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; Malchevski, 1985; Nicolov, 1984; Crichlow, 1989; Goranov et al., 1990; Ganter et al., 1993; Nicolov & Kunev, 1993b; Yoomm & Kim, 1993; Nicolov & Groseva, 1993; Nicolov, 1996)). Thus, there is a lot of information for their toxicodynamics, pathogenesis and diagnostics.

The dominating sign in acute nitrate poisoning is methaemoglobinaemia, that is

responsible for the lethal issue after hour 4 of the intoxication. The severe metabolic disorders in ruminants due to ingestion of rations containing excessive amounts of easily digested carbohydrates are manifested by hyperglycamia and lactacidaemia (Dirksen, 1970; Ganter et al., 1993). 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 (Nicolov, 2000) upon changes in clinical parameters in sheep receiving toxic doses of nitrates

and saccharose we found out that the character of methaemoglobinaemia was changed. It appeared later and was less expressed while the course of carbohydrate intoxication was similar to that in the independent poisoning.

There are no information about the changes in haematological and blood biochemical parameters of ruminants after the simultaneous ingestion of toxic doses of saccharose (easily digested carbohydrates) and nitrates. This fact motivated our study using a sheep model to evaluate the effect of toxic doses of these substances on haematological and blood biochemical status accompanying the corresponding clinical signs (Nikolov, 2000).

MATERIALS AND METHODS

Animals

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 endoparasites with 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.

Experimental design and treatments

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 rate of 0.65 g/kg b.w. (corresponding to 1/3 of the peroral LD₅₀ for rabbits) and 10.0 g/kg b.w. saccharose (about 10 times

higher than the therapeutic dose) 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.

Blood sampling

Heparinized, citrate and whole blood was sampled from *v. jugularis* 2 hours after the morning feeding for determination of haematological and biochemical parameters.

Blood samples were obtained in four consecutive days prior to the treatment (hours -72, -48, -24 and 0) and 2, 4, 6, 8, 10, 12, 24 and 48 hours thereafter.

Blood analysis

The following parameters were determined: haemoglobin (Hb), erythrocyte (RBC) and total leukocyte (WBC) counts – with an automated haemoanalyser (Sirano plus, Germany); methaemoglobin (MtHb) concentrations – according to the method of Derviz (1966); the differential WBC counts – via counting on a blood smear; the erythrocyte sedimentation rate (ESR) – according to Westergreen; blood sugar – with an automated analyser (Reflotron Manual, Germany) and serum lactate – with a commercial diagnostic kit (Boehringer, Germany).

Statistical analysis

The statistical significance of results was determined using the Student t-test at a level of significance of $p < 0.05$. Comparisons vs control alues were performed using zero values.

RESULTS

Hb concentrations in sheep from both experimental groups were within the physiological range prior to the treatments (at hours -72; -48; -24 and 0 – Fig. 1).

After the simultaneous application of both substances, this haematological parameter was initially decreased. At post treatment hour 4, Hb levels reached 7.5 ± 0.8 g/100 ml in group I and 7.1 ± 0.7 g/100 ml in

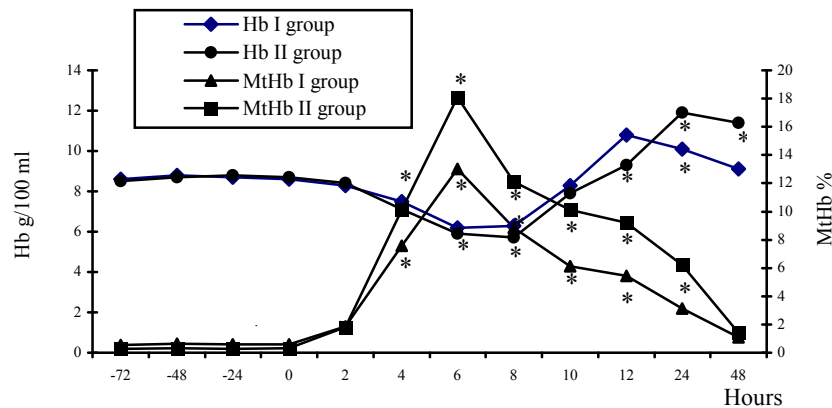


Fig. 1. Changes in blood haemoglobin and methaemoglobin concentrations in sheep simultaneously treated orally with 0.65 g/kg b.w. potassium nitrate and 10.0 g/kg b.w. saccharose (group I, n=6) or with 1 g/kg b.w. potassium nitrate and 20.0 g/kg b.w. saccharose (group II, n=6); Hb = haemoglobin; MtHb = methaemoglobin. * Statistically significant difference ($p < 0.05$) vs hour 0.

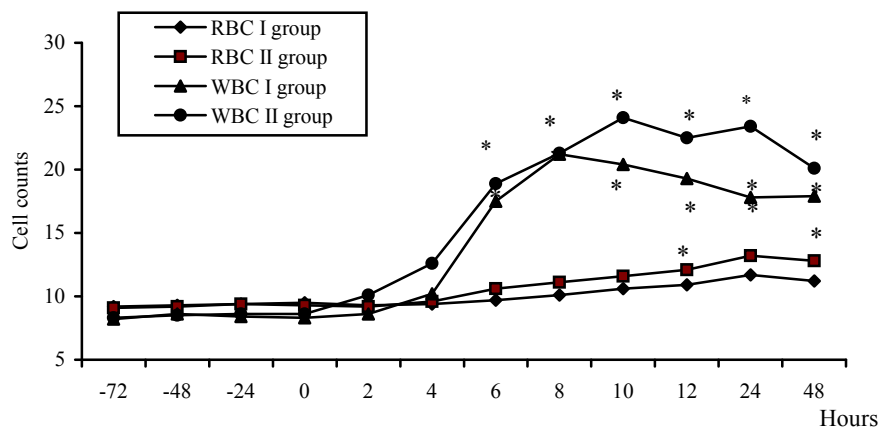


Fig. 2. Changes in red (T/l) and white (G/l) blood cell counts in sheep simultaneously treated orally with 0.65 g/kg b.w. potassium nitrate and 10.0 g/kg b.w. saccharose (group I, n=6) or with 1 g/kg b.w. potassium nitrate and 20.0 g/kg b.w. saccharose (group II, n=6); RBC = red blood cells, WBC = white blood cells. * Statistically significant difference ($p < 0.05$) vs hour 0. Y-axis: T/l or G/l.

Table 1. Changes in differential white blood cell counts 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).
* Statistically significant difference ($p < 0.05$) vs hour 0

Hours	Groups (I, n=6; II, n=6)	Differential WBC counts				
		Eosino- phils	Band neutro- phils	Seg- mented neutro- phils	Lympho- cytes	Mono- cytes
-72	I	1 ± 0.01	1 ± 0.02	37 ± 3.2	60 ± 0.0	1 ± 0.02
	II	2 ± 0.02	1 ± 0.02	38 ± 4.0	57 ± 0.0	2 ± 0.01
-48	I	2 ± 0.01	1 ± 0.02	36 ± 3.0	60 ± 0.0	1 ± 0.01
	II	1 ± 0.02	2 ± 0.01	35 ± 2.5	61 ± 0.0	1 ± 0.01
-24	I	1 ± 0.01	2 ± 0.02	37 ± 2.8	59 ± 0.0	1 ± 0.03
	II	1 ± 0.02	2 ± 0.03	39 ± 3.1	57 ± 0.0	1 ± 0.04
0	I	1 ± 0.01	1 ± 0.04	38 ± 4.2	60 ± 0.0	1 ± 0.05
	II	1 ± 0.03	1 ± 0.02	41 ± 4.3	55 ± 0.0	2 ± 0.02
2	I	0	1 ± 0.04	44 ± 4.1	54 ± 0.0	1 ± 0.04
	II	0	1 ± 0.05	39 ± 3.1	59 ± 0.0	1 ± 0.05
4	I	0	1 ± 0.05	45 ± 3.2	54 ± 0.0	0
	II	0	6 ± 0.04	46 ± 4.5	48 ± 0.0	0
6	I	0	1 ± 0.01	46 ± 4.0	33 ± 0.0*	0
	II	0	4 ± 0.03	57 ± 3.9	39 ± 0.0*	0
8	I	0	2 ± 0.04	49 ± 4.2*	49 ± 0.0	0
	II	0	2 ± 0.05	67 ± 3.8*	31 ± 0.0*	0
10	I	1 ± 0.01	1 ± 0.02	53 ± 4.4*	45 ± 0.0	0
	II	0	4 ± 0.02	69 ± 4.2*	27 ± 0.0*	0
12	I	0	1 ± 0.03	50 ± 3.1*	49 ± 0.0	0
	II	0	3 ± 0.03	73 ± 5.0*	24 ± 0.0*	0
24	I	0	1 ± 0.02	48 ± 2.3*	51 ± 0.0	0
	II	0	3 ± 0.02	60 ± 2.9*	37 ± 0.0*	0
48	I	0	1 ± 0.01	42 ± 2.2	57 ± 0.0	0
	II	0	1 ± 0.01	59 ± 2.4	40 ± 0.0	0

group II respectively. The peak decrease was observed at hours 6-8 (6.3 ± 0.2 g/100 ml in group I and 5.7 ± 0.4 g/100 ml in group II, $p < 0.05$). Afterwards, Hb concentrations increased and at hours 24-48 were higher than the initial ones.

MtHb percentages showed however more significant changes (Fig. 1). Prior to the treatment with nitrates and saccharose,

they were 0.25%-0.45% of total Hb. Following the application of both substances, MtHb increased statistically significantly as early as hour 4 and the peak levels were observed at hour 6 ($9.1 \pm 0.3\%$ for group I and $18.1 \pm 1.2\%$ for group II). Then, MtHb percentages decreased till the end of the experiment, but did not reach the physiological ranges in neither group.

The dynamics of change of RBC and WBC was unilateral (Fig. 2). RBC counts were increased and at hour 24 they reached 11.7 ± 0.2 T/l ($p < 0.05$) in group I and 13.2 ± 0.3 T/l ($p < 0.05$) in group II. The total WBC counts were increased in the early hours of the study. At hour 8, they were elevated to 21.2 ± 2.2 G/l in group I and 24.1 ± 3.1 G/l in group II ($p < 0.05$). The RBC and WBC counts were not normalized until the end of the experiment.

The differential WBC counts were characterized with a clear neutrophilia without a left shift, aneosinophilia (between hours 6-24) and lymphopenia (Table 1). The ESRs were lower. At the beginning, they were 17.7 mm/h and 17.1 mm/h in group I and II, respectively. Twelve hours after the treatment they fell to 12.5 mm/h and 10.3 mm/h, respectively. Similar changes were observed in ESR 2 and 24 hours following blood collection.

Blood sugar concentrations at baseline (hours -72, -48, -24 and 0) were within the normal range (2.73 ± 0.1 – 2.93 ± 0.2

mmol/l, Fig. 3). The combined nitrate-carbohydrate intoxication resulted in elevation at hour 4 in animals from both groups (4.2 ± 0.1 – 4.7 ± 0.4 mmol/l compared to initial levels). In group I, treated with lower doses of nitrate and saccharose, blood sugar was significantly increased ($p < 0.05$) at hour 8 (5.1 ± 0.3 mmol/l) and persisted elevated up to post treatment hour 12 (5.6 ± 0.3 mmol/l). Those concentrations remained higher up to hour 24 as well. The changes in the second group were similar with peak levels at hour 12 (6.2 ± 0.4 mmol/l, $p < 0.05$). By hour 48, this biochemical parameter was close to initial values.

The blood lactate content varied close to the normal range before the nitrate + saccharose treatment (2.1 ± 0.1 – 2.4 ± 0.1 mmol/l, Fig. 4). During the whole experimental period, lactate concentrations increased continuously. For group II, the levels reached 4.0 ± 0.2 mmol/l at hour 4 ($p < 0.05$). At hours 10 and 12 they raised up to 8.2 ± 0.4 and 8.4 ± 0.5 mmol/l respectively ($p < 0.05$) and persisted at those levels up to post treatment hour 24. At hour

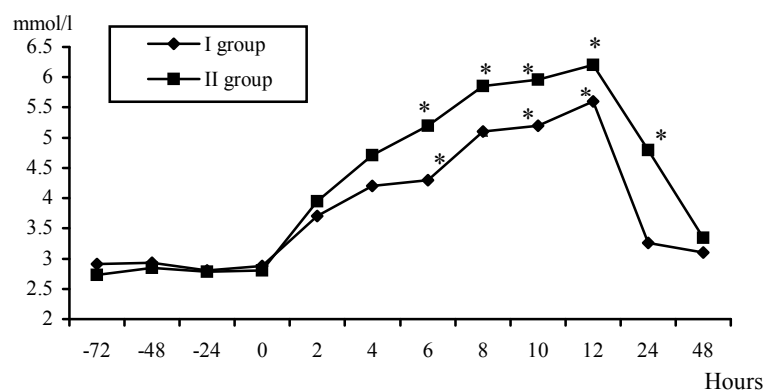


Fig. 3. Changes in blood sugar concentrations in sheep simultaneously treated orally with 0.65 g/kg b.w. potassium nitrate and 10.0 g/kg b.w. saccharose (group I, $n=6$) or with 1 g/kg b.w. potassium nitrate and 20.0 g/kg b.w. saccharose (group II, $n=6$). * Statistically significant difference ($p < 0.05$) vs hour 0.

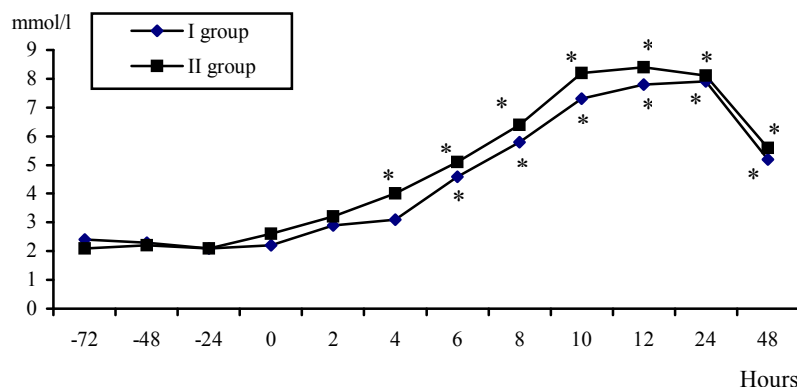


Fig. 4. Changes in blood lactate concentrations in sheep simultaneously treated orally with 0.65 g/kg b.w. potassium nitrate and 10.0 g/kg b.w. saccharose (group I, n=6) or with 1 g/kg b.w. potassium nitrate and 20.0 g/kg b.w. saccharose (group II, n=6). * Statistically significant difference ($p < 0.05$) vs hour 0.

48 the levels were still significantly increased (5.6 ± 0.5 mmol/l, $p < 0.05$). The sheep from group I showed a statistically significant elevation of blood lactate at hour 6 (4.6 ± 0.3 mmol/l) and at subsequent samplings. The dynamics of changes in group II was similar to that of group I between hours 10 and 24 (Fig. 4).

DISCUSSION

The haematological and biochemical changes in sheep treated with toxic doses of nitrates and saccharose were indicative for severe systemic metabolic and haemodynamic disorders. The simultaneous ingestion of both substances in such toxic doses results in degradation in rumen via independent biochemical pathways up to hour 2, without any interference (Nikolov, 2000). Nitrates are reduced to nitrites from nitrate reductase producing bacteria in rumen. One part of the latter are metabolized to hydroxylamine and ammonia utilized from forestomach microflora for synthesis of bacterial and protozoa proteins. Nitrites absorbed in blood circula-

tion oxidize haemoglobin Fe^{2+} to Fe^{3+} i.e. Hb becomes haemoglobin or MtHb (Alexander, 1972; Korzeniowski et al., 1980; Boermans, 1990). Thus, the increased MtHb percentages, observed in our experiments in the early hours following the intoxication (hours 4-6) could be explained by this process. It is known that if blood MtHb is up to 20% from total haemoglobin, the RBC enzyme reduction systems (methaemoglobinreductases) are fully reducing MtHb into Hb and therefore, clinical manifestations of methaemoglobinaemia are not observed, as is our case (Buck et al., 1976; Nikolov, 1984). The reduction of nitrates in the forestomachs was present up to post treatment hour 4 and afterwards it ceased (Nikolov, 1984). The other amounts of non-reduced nitrate are preserved unchanged. A part of them passes in the other parts of the gastro-intestinal tract while another part is absorbed in blood and excreted with urine (Nikolov, 1984). This explanation is further supported by our results for blood MtHb concentrations (Fig. 1). They sug-

gest that a clinical methaemoglobinaemia is lacking. Similar assumptions were made after studies on the single nitrate intoxication (Buck et al., 1976; Nikolov, 1984; Yoom and Kim, 1993).

The toxic doses of easily digested carbohydrates are rapidly metabolized by rumen microflora to ethanol, carbon dioxide, volatile fatty acid, lactate and other metabolites. The lactate is formed in the biggest amount, followed by propionic and butyric acids, whose normal ratio is impaired (Crichlow, 1989; Nikolov and Kunev, 1993a; Angelov et al., 1994; Nicolov, 1996). Those metabolites are responsible for the early decrease of rumen pH (6.3–6.0) at post treatment hours 4–6 (Nicolov, 2000). Thus, there are prerequisites for inhibition of the viability of nitrate reductase producing bacteria in rumen and consequently, for inhibition of the transformation of nitrates into nitrites. Our data for blood Hb and MtHb concentrations could be interpreted supposing that MtHb percentages, increased during the early hours and accompanied by decreased Hb levels, correlate with the period of normal functioning of nitrate reductase producing bacteria. The opposite changes, observed after hour 8 could be related to inhibition of that functioning following decrease of rumen pH.

It is known that although nitrate reduction in rumen is being interrupted by the decreased pH, carbohydrate fermentation continues more rapidly and by hours 8–12 rumen pH was about 5.0 (Nicolov, 2000). There is evidence that rumen lactate is absorbed in blood and influences blood acid-base status. In the early hours, a compensated metabolic acidosis occurs but later (hours 6–24) it becomes decompensated (Angelov et al., 1994). Our results showed that lactate concentrations were significantly increased compared to

baseline and by hour 48 remained still higher. The hyperglycaemia, observed in both groups, (manifested earlier and persisting longer in group II) confirms the fact that the excessive feeding of ruminants with easily digested carbohydrates leads to blood sugar elevation due to absorption of carbohydrate metabolites resulting from rumen fermentation processes. Those metabolites are further utilized as energy source. At the same time they activate glycogenolysis via a hormonal mechanism, and thus contribute for hyperglycaemia (Malchevski, 1985; Nicolov and Kunev, 1993b; Nicolov and Groseva, 1993; Nicolov, 1996).

CONCLUSIONS

Our studies upon the haematological and biochemical changes in sheep following ingestion of toxic doses of nitrates and easily digested carbohydrates, performed for the first time, showed that the simultaneous effect of both substances was manifested by severe haemodynamic and biochemical systemic changes, compensated by hour 48. The primary effects were due to the influence of carbohydrate rumen fermentation products that had a negative impact on systemic homeostasis. The increased Hb level, RBC and WBC counts, the delayed ESR resulted from the influence of easily digested carbohydrates on motility, secretory and microbiological functions of rumen. The simultaneous effects of carbohydrates and nitrates were the cause for lactacidaemia and hyperglycaemia. The toxic effect of nitrates was manifested in the early hours of the combined intoxication (up to hour 6) mainly by increasing MtHb percentages to 18.1% without clinical manifestations of methaemoglobinaemia. The rumen carbohydrate fermentation inhibited nitrate re-

duction and was therefore responsible for the lack of lethal issue un sheep with a clear methaemoglobinaemia.

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