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EFFECT OF NITRATE AND NITRITE POLLUTION ON SOME HAEMATOLOGICAL PARAMETERS OF RABBITS

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Haematological studies in rabbis fed on nitrates/nitrites revealed erythrocytopenia, leukocytopenia, decreased haemoglobin and lowered erythrocytic sedimentation rate up to 20 to 30 days postfeeding, showing an upward trend thereafter. The differences in various erythrocytic counts and erythrocytic sedimentation rates remained highly significant among rabbits fed nitrate/nitrite than those not fed nitrate/nitrite.
Key words: haematology, nitrates, nitrites, pollution, rabbits

INTRODUCTION

About 79.02% free molecule nitrogen (N_2) is present in our atmosphere which is inert chemically and can be utilized directly by only a few organisms. Nitrogen is taken up mostly in the form of nitrate, although ammonia is also utilized by some organisms (Nawaz, 1980). Maximum safe level of nitrate in water for livestock is less than 100 ppm. Although more than 500 ppm nitrates are required to produce acute poisoning. Death may result from 15000 ppm nitrate in the total diet. Nitrite is almost 10 times toxic than nitrates (Louis and Doull, 1975). To cope with the increasing demand of food for human beings, heavy use of pesticides and fertilizers also increased the nitrate and nitrite pollution. A variety of chemicals, being less toxic themselves but can be transformed into more toxic compounds or their derivatives which are not only mutagenic but can also be powerful carcinogens (Robertset al., 1990). Nitrate is one of them. It itself is not so toxic but it can be reduced metabolically to nitrite, which can act as a carcinogen (Butler and Jones, 1973).

Environmental and physiological conditions which influence nitrate assimilation in higher plants are light, drought, mineral nutrition, hormonal treatment, plantage and genetic composition. Nitrate accumulation is associated with deficiencies of Ca, Mg, Fe and K. Nitrate also induces the development of nitrate and nitrite reductase (Butler & Jones, 1973). Non-specific clinical symptoms associated with the nitrate toxicity are declining weight gain, loss of appetite, decreased milk production and digestive and respiratory disorders. The present study was, therefore, designed to study some clinical and haematological parameters in rabbits to ascertain the impact of nitrate and nitrite pollution.

MATERIALS AND METHODS

Seventy-two female rabbits of almost the same body weight and age were randomly divided into four groups designated as A, B, C and D. Each treatment was replicated three times in a factorial experiment. Animals of each group were caged separately. All the rabbits were fed green fodder and gram pulse. The animals of groups A and B were each fed sodium nitrate and sodium nitrite, respectively mixed in gram pulse @ 20 mg/kg body weight for a period of 40 days. Underground water sample was collected from Razaabad, Faisalabad and examined for nitrate and nitrite contents following A.O.A.C. (1984). For group C the same polluted water was used for soaking of gram pulse and fed to the rabbits. Group D rabbits served as control and were given the pulse soaked in water from WASA, Faisalabad water supply, having admissible levels of nitrates and nitrites. The animals were examined daily for any abnormality in their behaviour throughout the experimental period. Body weights of all the rabbits were recorded daily. Haematological studies comprising erythrocyte count, leukocyte count and erythrocyte sedimentation rate were made following Benjamin (1985). Haemoglobin was estimated by Sahil's comparison method. The data were subjected to statistical analysis using MSTAT package of computer.

RESULTS AND DISCUSSION

Clinical Studies: The oral administration of nitrite, nitrate and polluted water to rabbits caused a decrease in their weight gain in the first two weeks of feeding followed by a slight but progressive increase in body weight till the expiry of experiment (Table I). The sudden cessation of growth may be attributed to acute onset of alterations of metabolic pathway of biological system as the nitrite and nitrate have been reported to result in depletion of vitamin A and E in the body (Bruning and Kaneene, 1993). Manabu et at. (1983) reported that the ATPase activities are influenced by $NaNO_2$, but the increase in growth may be a feature of nitrate tolerance manifestation developed by repeated use of these agents. However, the weight gain in subsequent days was not of profound magnitude which may be assigned to poor retention of protein.

The rabbits of groups A, Band C showed various severe clinical signs such as mild to severe diarrhoea, frequent urination and vomit ion just after the feeding of nitrates, nitrites and polluted water. These manifestations might be

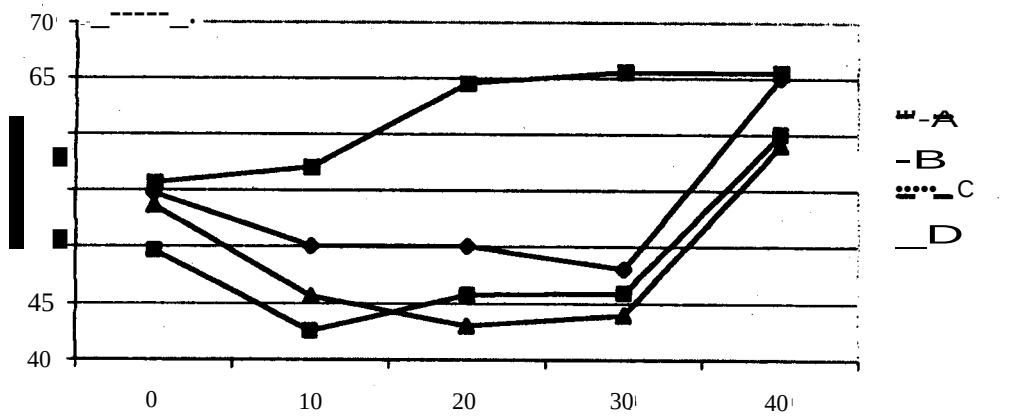
due to ill effects of these chemicals on the mucous membrane of gastrointestinal tract resulting in an increased peristaltic movements. Increased frequent urination might be due to diuretic effect and irritation caused to the urinary bladder by these toxic compounds. Bruning and Kaneene (1993) reported similar gastrointestinal tract disturbances and decreased food consumption produced by nitrate and nitrite. Dilatation of pupils, abdominal pain and convulsions were also observed in many of the affected rabbits. Cyanosis might have been due to higher level of methaemoglobin. Nervous signs were most probably due to the absorption of these chemicals from gastrointestinal tract, inability of the hepatic tissues to detoxify and ultimately disturbance of nervous system.

Table 1. Effect of nitrites/nitrates on body weight (Gram; mean ± SE) of rabbits

Groups	Days postfeeding				
	0	10	20	30	40
A	1047.2± 1.12	1003.8±0.98	917.1± 1.20	1007.9±1.12	1029.1±1.23
B	1030.7±1.05	1104.8± 1.17	1117.6±0.95	1179.1± 1.31	1119.9± 1.16
C	1033.2±1.08	1200.9± 1.03	1102.1±1.03	1247.1± 1.08	1234.7±1.11
D	1035.6±1.16	1151.1±1.10	1424.05± 1.30	1476.2± 1.16	1513.25± 1.45

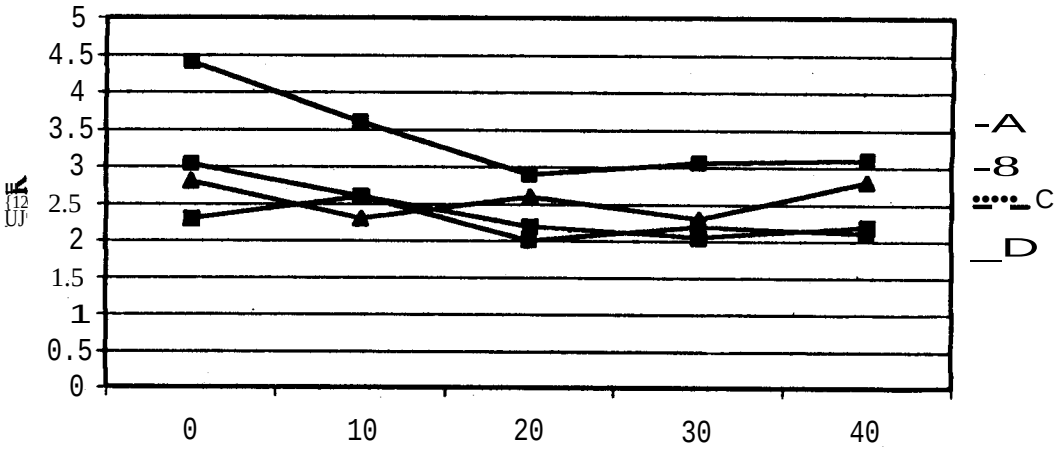
Table 2. Effect of nitrites/nitrates on erythroevtic count (million; mean ± SE) of rabbits

Groups	Days postfeeding				
	0	10	20	30	40
A	5.26±0.04	4.93±0.07	4.95±0.06	5.53±0.09	5.85±0.10
B	4.78±0.03	4.31±0.06	4.42±0.08	4.35±0.05	4.91±0.07
C	4.79±0.05	4.66±0.05	4.60±0.05	3.81±0.02	4.49±0.06
D	5.00±0.06	5.01±0.04	5.13±0.04	5.14±0.07	5.28±0.08



Days postfeeding

Fig.l. Effect of nitrate/nitrite on Hb (%) in various groups of rabbits



Days postfeeding

Fig.2. Effect of nitrate/nitrite on ESR in various groups of rabbits

Haematological Studies

i) Erythrocytic Count: The number of red blood cells decreased during the first two weeks and then slowly increased (Table 2) which is in line with the findings of Jirri et al. (1983). The initial decrease might be due to the effect of nitrites and nitrates on the Ca, Mg and ATPs activity of the cell membrane of RBCs. Dehydration due to diarrhoea and vomition might be another factor for this decrease in RBCs.

ii) Leukocytic Count: Leukocytic count dropped considerably in the nitrite and nitrate fed groups up to 30 days. Clinical manifestation consequent to the feeding of nitrate and nitrite included increased peristaltic movements and poor defensive mechanism probably due to decreased number of leukocytes. Later on, an increase in the number of leukocytes would have been due to adaptation of the rabbits and the stimulation of bone marrow activity. The rabbits in group C showed an increase in the number of leukocytes just after administration of polluted water. This increase might be due to enhanced bacterial content of polluted water. However, bacterial studies were not undertaken.

iii) Haemoglobin: Haemoglobin level fell in nitrite treated group (Fig. 1). A similar trend of haemoglobin decline was also reported by Kammerer and Sillart (1993). Nitrates in the body are converted into nitrite ions which can convert ferrous ions of the haemoglobin to ferric form, which is a stable oxidation product called methaemoglobin and is no more able to carry oxygen for respiratory functions resulting in tissue anoxia. Decrease in the number of erythrocytes which was observed just after the experimental feeding seems to be the most probable factor for the lowered haemoglobin level.

iv) Erythrocyte Sedimentation Rate (ESR): Nitrite and nitrate feeding resulted in decreased ESR (Fig.2). Jirri et al. (1983) also observed lowered ESR after feeding $NaN0_3$ at the level of 600 mill. Methaemoglobin and a decrease in the number of erythrocyte count might be the probable factors for the lowered ESR.

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