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NITRATE DETOXIFICATION OF DRINKING WATER BY ASCORBIC ACID IN GROWING RABBITS

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Abstract: This work was carried out to study the capacity of ascorbic acid for detoxification of drinking water nitrate in growing New Zealand White rabbits. Forty growing female rabbits were assigned to five groups (8 rabbits/group). The control animals (group 1) drank tap water without nitrate addition, those of group 2 drank water with 729 mg/l nitrate, while rabbits of groups 3, 4 and 5 drank water with the same nitrate level plus 100, 200 and 400 mg/l ascorbic acid respectively for seven weeks. The nitrate caused a significant (P<0.05) decrease in rabbit performance (feed and water intake, digestibility of nutrients and growth rate). Haemoglobin concentration, red blood cells count, total protein, albumin and globulin concentrations of animals drinking water with added nitrate decreased significantly (P<0.05) but aspertate aminotransferase and alanine aminotransferase activities, blood urea and cholesterol concentrations increased significantly (P<0.05). Daily weight gain decreased significantly (P<0.05) in 5th, 6th and 7th weeks in rabbits treated with nitrate. The rabbits treated with nitrate had a significant (P<0.05) worsening of feed conversion efficiency during the last three weeks of the treatment. The addition of ascorbic acid at three different levels (100, 200 and 400 mg/l) caused significant (P<0.05) increase in rabbit performance and led to an improvement of the measured blood parameters. In general, the results indicated that the highest level of improvement was obtained by adding ascorbic acid at the rate of 200 mg/l. The study also showed that 729 mg/l nitrate in drinking water had a toxic effect on rabbits. Ascorbic acid supplementation, especially at a level of 200 mg/l, was efficient and can be considered as a practical solution for nitrate detoxification of drinking water in growing rabbits.

Key words: rabbits, nitrate, water, ascorbic acid, growth performance, digestibility, blood parameters.

INTRODUCTION

Water sources can be contaminated by nitrates from barn and feedlot runoff, silage juice, nitrogen fertilizers, drainage and water effluents (Cheeke and Shull, 1985; El-Darawany *et al*., 1994; Wahaab and Badawy, 2004). In Egypt, there have

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been many illnesses as a result of drinking water polluted with high levels of nitrates, such as high methaemoglobin content (Selim, 1994) and urinary bladder and oral cancers (El-Aaser *et al.,* 1982; Badawi *et al.,* 1998). Hirneth and Classen, (1984) reported that the addition of ascorbic acid (vitamin C) decreased nitrite and methaemoglobin in the plasma of female rats fed a diet containing 5% $NaNO_3$. Also Men'kin *et al*. (1990) reported that the addition of ascorbic acid at twice the required level in the diet of laying hens containing 0.5% KNO_3 or 1% KNO_2 increased the weight of eggs and improved feed conversion efficiency, in comparison with the addition of ascorbic acid at the recommended level.

Our previous research (Bassuny *et al.,* 2004) on male growing rabbits indicated that a high level of nitrate (729 mg/L) in drinking water caused a significant reduction in feed intake, digestibility of nutrients, daily weight gain and also caused a serious change in blood components. However, until authors' knowledge, no scientific data are available on the capacity of ascorbic acid to detoxify nitrates in rabbits' drinking water or feed. This research was carried out to evaluate both the efficacy and the level of ascorbic acid required to minimise the toxicity of nitrates in rabbits' drinking water.

MATERIALS AND METHODS

This work was carried out in the Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt, in March and April 2004. Forty growing New Zealand White female rabbits were assigned to five groups (8 rabbits/ group) at 8 weeks of age. Animals were housed in individual cages under the same managerial, hygienic and environmental conditions. Rabbits of the 1st group (control) drank tap water without nitrate addition (control group), the 2nd group (nitrate) drank water supplemented with 1000 mg/L sodium nitrate, i.e. 729 mg/L nitrate, while rabbits of the 3rd, 4th and 5th groups drank water with the same level of nitrate plus 100, 200 and 400 mg/L ascorbic acid respectively for seven weeks. Ascorbic acid at 20% (United Co. for Chem. & Med. Prep., Egypt) was included at 0.5, 1.0 or 2.0 g/

Table 1: Chemical composition (%DM) of the experimental diet

Dry matter, %	90.0
Organic matter	89.9
Crude protein	16.7
Crude fibre	16.0
Ether extract	3.9
Nitrogen free extract	53.3
Ash	10.1
Nitrate	0.044

L to obtain 100, 200 or 400 mg/L ascorbic acid. Fresh water with the above-mentioned treatments was available all day and water intake was recorded daily. Tap water without nitrate addition had 1.2 mg/L nitrate. All animals were fed a pelleted commercial diet *ad libitum* (El-Morshedy Factory, Meetghamr, Dakahlia Governorate) for growing rabbits. The chemical composition of the diet is shown in Table 1. Feed intake and weight of the animals were recorded weekly. During the last week of the experiment, feed intake was recorded daily and faeces were collected from 3 rabbits for a digestibility test. At the end of the experiment, 3 rabbits from each treatment were slaughtered and the liver, kidneys, heart and lungs were removed from the body and subjected to post mortem examination. Blood samples were collected at slaughter in tubes containing heparin to estimate blood parameters. Hemoglobin and red blood cells counts were determined according to FRANKEL and REITMAN (1963) methods. Blood samples were centrifuged at 3,000 rpm for 15 minutes to obtain plasma. Plasma total protein, albumin, aspertate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol and urea were analyzed using commercial kits (Diamond Diagnostics Company, Egypt). The caecal bacterial count was determined using a haemocytometer according to SINGLETON and SAINSBURG (1981). Approximate analyses of feed and faeces were determined according to A.O.A.C. (1980). Nitrate was determined in diet and water using the Kjeldahl and Brucine methods, respectively, according to A.O.A.C. (1980). Data from the experiment were

statistically analyzed by one-way analysis of variance (Snedecor and Cochran, 1982). Differences between treatment means were tested according to Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Feed and water intake

The feed and water intake of the animals drinking water with nitrate presented lower values than that of the control animals throughout the experiment, but significant (P<0.05) differences were detected only in the 1st and 2nd week for feed and in the 1st week for water intake (Table 2). The decrease in feed and water intake after the 2nd week was not significant, possibly due to the animals becoming adapted to the nitrate (Kvasnicka and Krysl, 1996). These results agree with those obtained by Mahboob *et al.* (2001) and Bassuny *et al*. (2004). The decrease in feed and water intake may be due to decreased nutrient digestibility and abdominal pain (Mahboob *et al*., 2001; Bassuny *et al.,* 2004). The different levels of ascorbic acid (100, 200 and 400 mg/L) caused a significant (P<0.05) increase in feed intake during the 1st and 2nd week and water intake the 1st week only. Generally, the ascorbic acid groups showed daily feed intake similar to the control group throughout the duration of the experiments.

Digestibility, nutritive values and caecal bacterial count

The data presented in Table 3 show a significant (P<0.05) reduction in the digestibility of dry matter, organic matter, crude protein and crude fibre as well as in the caecal bacterial count of the animals drinking water with nitrate, compared with the control group. These results agree with those reported by Bassuny *et al*. (2004). The decrease in nutrient digestibility induced by nitrate may be due to diarrhoea, impairment of liver metabolism, mitochondrial dysfunction, reduction of the absorptive and secretive functions of intestinal mucosa and programmed cell death (Pfeifer and Weber, 1979; Zraly *et al*., 1997; Gow *et al*., 1998; Grudinski, 1998; Guzik *et al*., 2000; Mahboob *et al*., 2001). The decreased digestibility of crude fibre may be due to the significant (P<0.01) reduction in the caecal bacterial population (Table 4), since the conversion of nitrate to nitrite and nitric oxide has a potential

Table 2: Feed, water and nitrate intake.

Week	Control	Nitrate	Nitrate + 100 mg/l vit. C	Nitrate + 200 mg/l vit. C	Nitrate + 400 mg/l vit. C
Feed intake (g/d)					
1	104 ± 5^{a}	74 ± 6^{b}	102 ± 5^{a}	105 ± 6^{a}	105 ± 22^{a}
2	99 ± 8^{a}	79 ± 7^{b}	110 ± 4^{a}	109 ± 5^{a}	94 ± 4^{ab}
3	100 ± 3	95 ± 6	105 ± 7	114 ± 7	95 ± 5
4	119 ± 3	107 ± 5	126 ± 7	121 ± 2	120 ± 6
5	113 ± 7	112 ± 6	119 ± 8	113 ± 8	124 ± 6
6	122 ± 5	120 ± 5	123 ± 8	126 ± 8	120 ± 7
7	125 ± 4	119 ± 5	123 ± 9	127 ± 6	122 ± 7
Average	112 ± 4	101 ± 7	115 ± 4	116 ± 3	111 ± 5
Water intake (ml/d)					
1	243 ± 18^{ab}	184 ± 23^{c}	227 ± 19^{bc}	213 ± 19^{bc}	290 ± 12^{a}
2	243 ± 16	172 ± 12	204 ± 19	216 ± 31	227 ± 22
3	217 ± 21	199 ± 17	196 ± 8.0	214 ± 24	235 ± 18
4	209 ± 20	223 ± 24	244 ± 14	237 ± 23	267 ± 9.0
5	263 ± 10	229 ± 28	247 ± 9.0	250 ± 51	275 ± 10
6	258 ± 15	263 ± 18	263 ± 9.0	250 ± 31	301 ± 15
7	255 ± 9	254 ± 18	257 ± 9.0	249 ± 22	289 ± 12
Average	241 ± 8.0	218 ± 13	234 ± 10	233 ± 7.0	269 ± 11
Nitrate intake (mg/d)					
1	42 ± 3^{d}	164 ± 12^{c}	206 ± 10^{b}	198 ± 8^{b}	254 ± 10^{a}
2	40 ± 4^{c}	157 ± 10^{b}	193 ± 12^{a}	201 ± 8^{a}	195 ± 12^{a}
3	40 ± 4^{c}	183 ± 10^{b}	185 ± 14^{b}	202 ± 10^{ab}	209 ± 18^{a}
4	48 ± 3^{d}	206 ± 14^{c}	228 ± 17^{ab}	222 ± 10^{b}	243 ± 12^{a}
5	45 ± 5^{d}	212 ± 10^{c}	228 ± 8.0^{b}	228 ± 8^{b}	250 ± 12^{a}
6	49 ± 3^{c}	240 ± 15^{b}	241 ± 12^{b}	233 ± 10^{b}	268 ± 10^{a}
7	50 ± 4^{c}	234 ± 10^{b}	237 ± 10^{b}	232 ± 12^{b}	260 ± 8^{a}
Average	45 ± 3^{d}	199 ± 10^{c}	217 ± 7^{b}	216 ± 5^{b}	240 ± 8^{a}

Means within a row with different superscripts differ. ($P<0.05$).

antimicrobial effect on the cellulolytic caecal bacteria (Spears *et al*., 1977; Marais *et al.*, 1988; Baimatov, 1991; Xu and Verstraete, 2001).

The addition of ascorbic acid at all levels significantly increased ($P<0.05$) both the digestibility of all nutrients and the bacterial count (Table 3). This may be due to the antioxidant activity of vitamin C, which has an inhibitory effect on the conversion of nitrate to nitrite and nitric oxide, since it is known that nitrite is eight times more toxic than nitrate (Cheeke and Shull, 1985).

Total digestible nutrient and digestible protein intake (g/d) were significantly reduced ($P<0.05$) by nitrate treatments (Table 3). Similar results were reported by Bassuny *et al*. (2004). These results were caused by the decreased feed intake and digestibility coefficients. So the addition of ascorbic acid at all levels significantly increased ($P<0.05$) the nutritive value and intake of feed.

Blood parameters

The haemoglobin concentration and red blood cells count (RBCs) decreased significantly ($P<0.05$) in animals drinking water with nitrate (Table 4). These results are in agreement with those obtained by Kammerer and Siliart (1993), Mahboob *et al*. (2001) and Bassuny *et al*. (2004). This change in haemoglobin concentration as compared to the control animals could be explained, firstly, by an increase in the activity of the endothelial heme oxygenase by nitric oxide, which degrades heme to carbon monoxide and biliverdin (Foresti *et al.,* 1997), and secondly, by the formation of peroxynitrite, which reduces the production of the rabbit tissue factor, a primary initiator of physiological blood coagulation (Nielsen and Crow, 2004). Nitric oxide produced in the lung causes injury to pulmonary cells (Gow *et al*., 1998).

Total protein, albumin and globulin concentrations were significantly reduced ($P<0.05$) by nitrate (Table 4). These results agree with those reported by Bassuny *et al.* (2004). The decrease in plasma total protein, albumin and globulin concentrations may be due to formation of nitric oxide or peroxynitrite, which oxidises proteins and lipoproteins (Gow *et al.*, 1998; Guzik *et al*., 2000), impairs liver metabolism

Table 3: Apparent digestibility coefficients and caecal bacterial count.

	Treatments				
	Control	Nitrate	Nitrate + 100 mg/l vit. C	Nitrate + 200 mg/l vit. C	Nitrate + 400 mg/l vit. C
Apparent digestibility coefficients (%):					
Dry matter	74.4 ± 1.9[a]	58.2 ± 2.8[b]	68.2 ± 2.7[a]	70.1 ± 2.3[a]	67.2 ± 3.6[a]
Organic matter	77.3 ± 3.9[a]	61.7 ± 2.5[b]	70.8 ± 2.4[a]	72.3 ± 2.0[a]	69.7 ± 3.3[ab]
Crude protein	76.3 ± 1.8[a]	67.4 ± 2.1[b]	76.7 ± 1.8[a]	78.0 ± 4.3[a]	79.4 ± 3.7[a]
Crude fibre	43.1 ± 4.5[a]	18.3 ± 3.1[b]	38.5 ± 2.5[a]	43.3 ± 2.2[a]	38.8 ± 3.4[a]
Ether extract	89.2 ± 3.6	86.9 ± 1.5	84.5 ± 4.4	90.3 ± 4.1	84.0 ± 1.7
Nitrogen free extract	77.3 ± 3.1	73.1 ± 1.4	77.6 ± 4.0	77.2 ± 1.5	78.0 ± 1.7
Total digestible nutrient intake (g/d)	91.1 ± 3.4[a]	72.6 ± 0.8[b]	87.1 ± 5.3[a]	79.8 ± 4.5[ab]	84.4 ± 8.1[ab]
Digestible crude protein intake (g/d)	16.8 ± 0.3	13.6 ± 0.5	16.4 ± 0.6	15.0 ± 0.9	16.7 ± 1.7
Caecal bacterial counts (cells×10^9/ml)	5.2 ± 0.2[a]	1.8 ± 0.1[c]	4.0 ± 0.3[b]	4.3 ± 0.2[b]	4.2 ± 0.1[b]

Means within a row with different superscripts differ. ($P<0.05$).

(ZRALY *et al*., 1997) and impairs kidney functions (PFEIFER and WEBER, 1979). The decreased globulin concentration caused by nitrate treatment may indicate an immunodepressive response (ATEF *et al*., 1991). The activity of AST and ALT were significantly (P<0.05) increased by nitrate treatment (Table 4). Similar results in rabbits and rats were also reported by POPOV *et al*. (1996) and BASSUNY *et al*. (2004). The increase in AST and ALT indicates hepatocyte necrosis (ALLIS *et al.,* 1990).

Nitrate caused an increase in blood urea (Table 4). Cholesterol concentration was significantly higher (P<0.05) in animals that drank water with nitrate. Hypercholesterolemia indicates vascular disease and endothelial dysfunction caused by nitric oxide and superoxide (GUZIK *et al*., 2000). The above changes in blood parameters indicate liver and kidney damage and immunodepression as a result of nitrate treatment.

The addition of ascorbic acid improved all the blood parameters measured. Similar results were reported by SELIM (1994) who observed that ascorbic acid reduced

Table 4: Blood parameters.

	Treatments				
	Control	Nitrate	Nitrate + 100 mg/l vit.C	Nitrate + 200 mg/l vit.C	Nitrate + 400 mg/l vit.C
Haemoglobin (g/dl)	8.87 ± 0.44^{a}	7.73 ± 0.24^{b}	8.57 ± 0.08^{a}	8.60 ± 0.11^{a}	9.20 ± 0.76^{a}
RBC count(10^{6}/ml)	6.38 ± 0.21^{a}	5.54 ± 0.06^{b}	5.81 ± 0.49^{ab}	6.71 ± 0.27^{a}	7.12 ± 0.64^{a}
AST (u/l)	35.67 ± 2.33^{b}	43.33 ± 1.45^{a}	36.00 ± 2.02^{b}	34.00 ± 2.08^{b}	33.00 ± 1.72^{b}
ALT (u/l)	23.67 ± 2.84^{b}	33.33 ± 2.65^{a}	24.33 ± 0.66^{b}	22.67 ± 1.45^{b}	24.67 ± 0.34^{b}
Total protein (g/dl)	6.67 ± 0.33^{a}	4.50 ± 0.29^{c}	5.33 ± 0.17^{bc}	5.67 ± 0.33^{b}	5.67 ± 0.17^{b}
Albumin (g/dl)	4.21 ± 0.12^{a}	3.80 ± 0.12^{c}	4.03 ± 0.01^{bc}	4.13 ± 0.01^{b}	4.12 ± 0.21^{b}
Globulin (g/dl)	2.46 ± 0.24^{a}	0.70 ± 0.17^{c}	1.31 ± 0.17^{bc}	1.54 ± 0.33^{b}	1.36 ±0.20^{bc}
Cholesterol (mg/dl)	70.76 ± 0.40^{c}	91.97 ± 4.21^{a}	71.52 ± 1.86^{c}	71.97 ± 2.78^{c}	81.39 ± 0.90^{b}
Urea (mg/dl)	30.65 ±1.73^{b}	36.41 ±0.93^{ab}	30.45 ± 2.37^{b}	30.63 ± 0.71^{b}	30.86 ± 1.08^{b}

RBC: red blood cells, AST: aspartato aminotrasferase, ALT: alanine aminotrasferase.
Means within a row with different superscripts differ. (P<0.05).

methaemoglobin and increased haemoglobin concentrations in humans who drank water naturally polluted with nitrate. Additionally, Hirneth and Classen (1984) reported that vitamin C inhibited the production of plasma nitric oxide and methaemoglobin in female rats fed with a diet containing 5% $NaNO_3$. The improvement caused by ascorbic acid may be due to stimulation of the immune system, which results in an increased corticosterone concentration (Rama Rao *et al*., 2002) and reduced endogenous formation of N-nitrosodimethylamine and N-nitrosopiperdine from nitrate (Vermeer *et al*., 1999).

Growth rate and feed conversion

The daily weight gain of rabbits that drank water with nitrate decreased significantly ($P<0.05$) in comparison with that of the control animals during the total period (Table 5). However, the highest decrease in body weight gain occurred during the first two weeks. Thereafter the decrease was significant but not so marked. These results may be due to the animals' adaptation with time (Kvasnicka and Krysl, 1996). Similar results were reported by Bassuny *et al.* (2004). The decrease in weight gain may be due to the decrease in feed intake, as well as to the negative effects on metabolism and physiology described above. The addition of ascorbic acid at the levels used in this study caused an increase in weight gain. In particular, the weight gain of animals that drank water with nitrate plus 200 mg/L ascorbic acid was significantly higher than that of the animals that drank water with nitrate throughout the entire experiment.

The feed conversion (feed/gain) of animals drinking water with nitrate was significantly ($P<0.05$) different in comparison with that of the control group only in the 5th, 6th and 7th weeks of the trial. The average feed conversion for the total period did not differ significantly among the groups (Table 5). These results were similar to those reported by Bassuny *et al*. (2004) on male rabbits. The negative impact of nitrate on feed conversion may be due to decreased nutrient digestibility and its toxic effect on the absorptive and secretive functions of intestinal mucosa which affects cell maturation, differentiation and death (Grudinski, 1998).

Table 5: Growth performance.

Week	Treatments				
	Control	Nitrate	Nitrate + 100 mg/l vit. C	Nitrate + 200 mg/l vit. C	Nitrate + 400 mg/l vit. C
Live body weight (g)					
Initial	1037 ± 32	1099 ± 41	1099 ± 41	1058 ± 29	1106 ± 14
1	1192 ± 36	1208 ± 46	1208 ± 46	1200 ± 32	1265 ± 17
2	1350 ± 37	1301 ± 45	1301 ± 45	1355 ± 32	1395 ± 14
3	1513 ± 36	1438 ± 45	1438 ± 45	1515 ± 27	1538 ± 15
4	1673 ± 32	1570 ± 40	1570 ± 40	1675 ± 25	1678 ± 14
5	1838 ± 31^{a}	1707 ± 42^{b}	1707 ± 42^{b}	1839 ± 24^{a}	1818 ± 13^{a}
6	1990 ± 30^{a}	1827 ± 38^{b}	1827 ± 38^{b}	1990 ± 26^{a}	1960 ± 13^{a}
7	2149 ± 28^{a}	1945 ± 39^{b}	1945 ± 39^{b}	2145 ± 25^{a}	2105 ± 14^{a}
Weight gain (g/d)					
1	22.1 ± 0.4^{a}	15.6 ± 0.5^{c}	15.6 ± 0.5^{c}	20.4 ± 0.5^{ab}	22.7 ± 0.5^{a}
2	22.6 ± 0.5^{a}	13.2 ± 0.7^{c}	13.2 ± 0.7^{c}	22.1 ± 0.9^{a}	18.6 ± 0.7^{b}
3	23.1 ± 0.3^{a}	19.6 ± 0.8^{b}	19.6 ± 0.8^{b}	22.9 ± 1.2^{a}	20.4 ± 0.8^{b}
4	23.0 ± 0.4^{a}	18.9 ± 1.0^{b}	18.9 ± 1.0^{b}	22.9 ± 0.6^{a}	20.1 ± 0.4^{b}
5	23.6 ± 0.7^{a}	19.6 ± 1.0^{b}	19.6 ± 1.0^{b}	23.5 ± 0.5^{a}	20.0 ± 0.5^{b}
6	21.7 ± 0.4^{a}	17.1 ± 0.8^{b}	17.1 ± 0.8^{b}	21.6 ± 0.8^{a}	20.2 ± 0.6^{a}
7	22.7 ± 0.5^{a}	16.9 ± 0.5^{c}	16.9 ± 0.5^{c}	22.1 ± 0.6^{ab}	20.7 ± 0.5^{b}
Average	22.7 ± 0.2^{a}	18.7 ± 1.1^{d}	18.7 ± 1.1^{d}	22.2 ± 0.4^{ab}	20.4 ± 0.5^{bc}
Feed conversion (feed/gain)					
1	4.71 ± 0.18	4.75 ± 0.23	4.75 ± 0.23	5.16 ± 0.23	4.60 ± 0.12
2	4.38 ± 0.15	6.00 ± 0.27	6.00 ± 0.27	4.90 ± 0.18	5.08 ± 0.22
3	4.34 ± 0.13	4.83 ± 0.17	4.83 ± 0.17	4.97 ± 0.15	4.64 ± 0.19
4	5.17 ± 0.17^{c}	5.71 ± 0.18^{ab}	5.71 ± 0.18^{ab}	5.31 ± 0.17^{bc}	5.96 ± 0.28^{a}
5	4.78 ± 0.21^{c}	5.74 ± 0.13^{bc}	5.74 ± 0.13^{bc}	4.81 ± 0.24^{c}	6.19 ± 0.17^{a}
6	5.60 ± 0.18^{b}	7.01 ± 0.29^{a}	7.01 ± 0.29^{a}	5.86 ± 0.25^{b}	5.91 ± 0.11^{b}
7	5.50 ± 0.19^{b}	7.05 ± 0.31^{a}	7.05 ± 0.31^{a}	5.72 ± 0.13^{b}	5.89 ± 0.26^{b}
Average	4.93 ± 0.20	5.87 ± 0.12	5.87 ± 0.12	5.25 ± 0.16	5.47 ± 0.25

Means within a row with different superscripts differ. (P<0.05).

Table 6: Internal organs weight (% live weight).

	Treatments				
	Control	Nitrate	Nitrate + 100 mg/l vit. C	Nitrate + 200 mg/l vit. C	Nitrate + 400 mg/l vit. C
Liver	3.13 ± 0.07^{b}	3.75 ± 0.14^{a}	3.45 ± 0.10^{ab}	3.17 ± 0.09^{a}	3.25 ± 0.11^{a}
Heart	0.28 ± 0.04	0.33 ± 0.02	0.32 ± 0.03	0.29 ± 0.02	0.28 ± 0.06
Kidneys	0.78 ± 0.05	0.83 ± 0.02	0.81 ± 0.05	0.81 ± 0.02	0.81 ± 0.04
Lungs	0.70 ± 0.03^{b}	0.80 ± 0.03^{a}	0.77 ± 0.02^{ab}	0.72 ± 0.03^{b}	0.75 ± 0.02^{ab}

Means within a row with different superscripts differ. ($P<0.05$).

Mortality, organ weights and clinical signs of toxicity

Mortality rate (%) was zero during the whole experiment. Nitrate treatment increased the relative weight values (% of live weight) of internal organs, but the increase was significant ($P<0.05$) only for the liver and the lungs (Table 6). The colour of the lungs was pale and there was a small purulence on the right lung. These results may be due to death of the lung cells by nitric oxide or peroxynitrite (GOW *et al*., 1998; GUZIK *et al*., 2000). Blood colour was brown or chocolate, which may be attributed to increased methaemoglobin formation (CHEEKE and SHULL, 1985). Addition of ascorbic acid, especially at the level of 200 mg/L or more, diminished the effect of nitrate on internal organ weight and on the clinical symptoms of toxicity.

CONCLUSIONS

Nitrate in drinking water leads to a reduction in rabbit performance and feed efficiency and also to relevant changes in the blood components. The addition of ascorbic acid, especially at the level of 200 mg/L or more, was an efficient and practical method for the detoxification of nitrates in the drinking water of growing rabbits and produced no side effects.

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