

## Effect of potassium nitrate plus vitamin C in feed of rabbits on the some biochemical parameters

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### Summary

This study is conducted at investigating the effect of potassium nitrate and vitamin C in feed of the rabbits on the some biochemical parameters. Twenty eight adult New-Zealand rabbits were divided randomly into four groups (7 rabbits each), they were fed potassium nitrate and vitamin C for 16 weeks as follow: Group 1 (G<sub>1</sub>) fed potassium nitrate (168 mg/ kg B.W./ daily), group 2 (G<sub>2</sub>) fed potassium nitrate (168 mg/ kg B.W./ daily) and vitamin C (50 mg/ kg B.W./ daily), group 3 (G<sub>3</sub>) fed potassium nitrate (168 mg/ kg B.W./ daily) and vitamin C (100 mg/ kg B.W./ daily) and group 4 (G<sub>4</sub>) fed basal ration as control group. Blood were collected from heart at zero, eight, twelve and sixteen weeks. The results showed a significant increase (P<0.05) in cholesterol, triglyceride and blood nitrogen urea in the group that had fed potassium nitrate G<sub>1</sub> compared with G<sub>4</sub>, whereas the groups that were fed vitamin C with potassium nitrate showed a mild decrease compared with group potassium nitrate that had been fed potassium nitrate alone (G<sub>1</sub>). In conclusion, the feeding rabbits with potassium nitrate caused an increase in cholesterol, triglyceride and blood urea concentration in the serum, whereas the vitamin C ameliorates this effect.

**Keywords: Potassium Nitrate, Vitamin C, Rabbit, Biochemical parameters.**

### Introduction

The increase in the pollution of environment is a major and global problem, nitrate levels in water and food supplies have been increased during the last decades worldwide, the nitrate pollution has become a global concern, which may affect the food quality for daily use and threaten the human and animal health (1). Several studies reported that excessive nitrate intake causes many problems, such as methaemoglobinaemia (2-4). El-Wakf (5) recorded that other possible outcomes of prolonged nitrate exposure include cancer, via the bacterial production of N-nitroso compounds, hypertension, increased infant mortality, birth defects of central nervous system, spontaneous abortion, growth retardation, diabetes, respiratory tract infections, and changes to the immune system. Moreover, nitrate toxicity also causes an elevation in glucose, cholesterol, aspartate transaminase and alanine transaminase in serum rats (6). The using antioxidant vitamins, such as vitamins A, E and C, to enhance protection against chemical agent-induced toxicity have been widely reported (7 and 8). Vitamin C is known to be one of the potent antioxidants that is capable of protecting various biological systems against the toxic

effects of reactive species generated by different chemical agents (9-11), and shown to scavenge free radicals in the body (12 and 13). The aim of this study was to evaluate the effect of potassium nitrate and vitamin C in feed of the rabbits on the some biochemical parameters.

### Material and Methods

Twenty-eight adult New-Zealand rabbits were with an age of 10 - 14 months and weight 1850-2100gm. Rabbits were divided randomly into four groups, each group consist of 7 rabbits: Group 1 (G<sub>1</sub>): Rabbits of this group were fed ration I (Table, 1) which was supplemented with potassium nitrate (168 mg/ kg B.W./ daily) according to (14) as potassium nitrate treated group. Group 2 (G<sub>2</sub>): Rabbits of this group were fed ration II (Table, 1) which was supplemented with potassium nitrate (168 mg/ kg B.W./ daily) and vitamin C (50 mg/ kg B.W./ daily) according to (15). Group 3 (G<sub>3</sub>): Rabbits of this group were fed ration III (Table, 1) which was supplemented with potassium nitrate (168 mg/ kg B.W./ daily) and vitamin C (100 mg/ kg B.W./ daily) according to (15). Group 4 (G<sub>4</sub>): Rabbits of this group were fed ration number IV (Table, 1) as control group. During the experiment, the

blood were collected from heart at zero, eight, twelve and sixteen weeks, the spectrophotometric methods kits (Biolabo) were used to measure the serum cholesterol, triglyceride and blood urea nitrogen (BUN) in serum according to (16).

The data obtained were expressed as means ± standard error (SE) and subjected to statistical analysis using one-way analysis of variance (ANOVA) and Least significant differences (LSD) post hoc test was performed by using SPSS-20 (Statistical Packages for Social Sciences, version 20) (17).

**Table, 1: Different nutritional rations fed to different rabbit groups.**

Ration Component	Ration I	Ration II	Ration III	Ration IV
Corn	31 %	31 %	31 %	31 %
Barley	15 %	15 %	15 %	15 %
Soya	10 %	10 %	10 %	10 %
Bran	30 %	30 %	30 %	30.54 %
Hay	10 %	10 %	10 %	10 %
Animal protein	2.46 %	2.46 %	2.46 %	2.46 %
KNO <sub>3</sub>	0.54 %	0.54 %	0.54 %	-----
Vitamin C	-----	0.1 %	0.2 %	-----
Calcium	0.7 %	0.7 %	0.7 %	0.7 %
NaCl	0.3 %	0.3 %	0.3 %	0.3 %

Individual feed intake = 100±5 g  
 Convert factor of KNO<sub>3</sub> to NO<sub>3</sub> is 1.61

### Results and Discussion

The effect of potassium nitrate supplementation in feed of rabbits on the cholesterol levels are shown in (Table, 2). The cholesterol levels of all groups were non-significantly increased during the study period with the progression of experiment, while significant differences (P<0.05) were showed in last period between G<sub>1</sub> and G<sub>4</sub> at the sixteenth week. The highest triglyceride level was recorded in G<sub>1</sub> (118.60±8.21 mg/dl) and showed a significant increases (P<0.05) in G<sub>1</sub> as compared with groups that fed potassium nitrate + vitamin C (G<sub>2</sub> and G<sub>3</sub>) and the results showed a significant increase (P<0.05) in groups that fed potassium nitrate G<sub>1</sub>, G<sub>2</sub> and G<sub>3</sub> as compare with G<sub>4</sub> at the sixteenth week (Table, 3). The blood nitrogen urea concentrations are presented in (Table, 4). The results showed a significant increase (P<0.05) in G<sub>4</sub>, G<sub>3</sub> and G<sub>2</sub> as compare with G<sub>1</sub>. These elevated levels were observed on eighth and sixteenth week respectively.

The health risks of nitrate exposure have been widely evaluated in several vertebrates (18). Nitrate poisoning affects several biochemical parameters (19). The results revealed increased levels of cholesterol and triglyceride in serum of rabbits that were treated with nitrate. This might be due to effect of nitrate to the liver function (20). Liver plays an active important role in metabolism of cholesterol (21). Nitrate is a source of nitric oxide (NO) and other reactive oxygen as well as nitrogen species such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), peroxy nitrite (ONOO-) and superoxide anion (O<sub>2</sub>-), which disturbs the balance between pro-oxidants and antioxidants in favor of the former, resulting in oxidative stress (22 and 23). Nakano and Tomlinson (24) recorded that stressful stimuli elicit a rapid secretion of glucocorticoids and catecholamines from adrenal tissue. Both of these hormones are known to produce hyperglycemic response (25), this results in agreement with other authors (5, 19 and 25), who indicated that a high nitrate intake causes a significant increase in lipid profile.

**Table, 2: The effect of potassium nitrate and vitamin C as feed additive to the feed of rabbits on the serum cholesterol (mg /dl).**

Groups Time (week)	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>
Zero	49.74 ±4.72	50.60 ±5.63	53.98 ±6.01	55.36 ±8.25
8 <sup>th</sup>	51.82 ±7.36	52.15 ±3.52	49.02 ±4.22	62.84 ±7.23
12 <sup>th</sup>	64.53 ±5.11	61.21 ±2.88	59.82 ±2.65	60.17 ±8.33
16 <sup>th</sup>	69.06 ±2.64 A	63.68 ±3.23 AB	62.87 ±2.99 AB	60.91 ±6.23 B

The different capital letters refer to significant differences between different groups at (P<0.05).

The reduction in the levels of cholesterol and triglyceride in groups that fed potassium nitrate and vitamin C as compared with group that fed potassium nitrate alone, was due to the effect of vitamin C, which reduces nitrate toxicity by inhibiting endogenous nitrosation (1). Moreover, vitamin C is effective in the protection against oxidative damage in tissues and suppresses formation of nitrosamines (26), therefore, rabbits that fed vitamin C showed mild recovery from the nitrate toxicity,

whereas a 100 mg/kg B.W. level was more effective as compared to the 50 mg/kg B.W. level. Nitrate may cause multiple physiological impacts if the feed of animal have a high nitrate concentration (27).

**Table, 3: The effect of potassium nitrate and vitamin C as feed additive to the feed of rabbits on the serum triglyceride (mg/dl).**

Groups Time (week)	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>
Zero	74.28 ±8.14	70.53 ±6.18	72.59 ±9.24	74.81 ±5.28
8 <sup>th</sup>	99.85 ±6.95	89.14 ±5.82	80.08 ±9.13	84.14 ±9.59
12 <sup>th</sup>	97.51 ±8.71	96.85 ±6.12	93.80 ±4.03	82.55 ±7.65
16 <sup>th</sup>	118.60 ±8.21 A	102.20 ±4.15 B	95.89 ±5.69 B	80.36 ±6.98 C

The different capital letters refer to significant differences between different groups at (P<0.05)

**Table, 4: The effect of potassium nitrate and vitamin C as feed additive to the feed of rabbits on the serum urea (mg / dl).**

Groups Time (week)	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>
Zero	45.97 ±8.59	47.05 ±3.40	44.47 ±3.19	45.12 ±4.90
8 <sup>th</sup>	58.45 ±3.22 A	57.26 ±3.35 A	56.20 ±4.22 A	48.85 ±3.81 B
12 <sup>th</sup>	58.24 ±3.34 B	55.56 ±4.10 AB	55.20 ±3.20 AB	46.05 ±3.91 B
16 <sup>th</sup>	59.75 ±3.92 A	56.27 ±3.86 A	55.42 ±2.56 A	45.37 ±4.62 B

The different capital letters refer to significant differences between different groups at (P<0.05).

In the present study, exposure to nitrate caused elevation in urea concentration which obvious in groups that fed potassium nitrate, these may be explained by impaired kidney function that caused by nitrate toxicity (28). Nitrite effect on the process of absorption and reabsorption in the kidney tubules (5), urea is the principal product of protein catabolism, the reduction in total protein in animals exposed to environmental pollutants could be attributed to changes in protein and free amino acid metabolism, such as reduced protein synthesis or increased proteolytic activity or degradation (29). Helal *et al.* (30) investigated that serum protein of rats decreased due to the toxic effect

of nitrite, while fast breakdown occurs, and increasing of nitrogen intake that came from potassium nitrate leads to an increase of urea in blood.

Conclusion, that the exposure for long period to potassium nitrate causes an increase in cholesterol, triglyceride and blood urea, whereas the vitamin C ameliorate this effect. Finlay, recommend that high levels of vitamin C (100 mg/kg B.W. daily) must be added to combat the toxic effect of potassium nitrate.

### References

1. Ward, M.; Kilfoy, B. and Weyer, P. (2010). Nitrate intake and the risk of thyroid cancer and thyroid disease. *Epidemiol.*, 21(3):389-395.
2. Sadeq, M.; Moe, CL.; Attarassi, B.; Cherkaoui, I.; El -Aouada, R. and Idrissi, L. (2008). Drinking water nitrate and prevalence of methemoglobinemia among infants and children aged 1-7 years in Moroccan areas. *Int. J. Hyg. Environ. Health.*, 211:546-5454.
3. Curley, E. M.; Flynn, M G. and Mc Donnell, K. P. (2009). Nitrate Leaching Losses from *Miscanusciganteus* Impact of Ground Water Quality, *J. Appl. Sci.*, 8(3):107-112.
4. Rodriguez-Estival, J.; Martinez-Haro, M.; Martin-Hernando, M. P. and Mateo, R. (2010). Sub-chronic effects of nitrate in drinking water on red-legged partridge (*Alectoris rufa*): Oxidative stress and T-cell mediated immune function. *Environ. Res.*, 110:469-475.
5. El-Wakf, A.; Hassan, H.; Elsaid, F. and El-Said, A. (2009). Hypothyroidism in male rats of different ages exposed to nitrate polluted drinking water. *Res. J. Med. Medical Sci.*, 4(2):160-164.
6. Messaadia, A.; Saka, S.; Krim, M.; Maida, I.; Aouacheri, O. and Djafer, R. (2013). Ginger-supplemented diet ameliorates ammonium nitrate-induced oxidative stress in rats. *12(40):5909-5916.*
7. Schafer, F. Q.; Wang, H. P.; Kelley, E.; Cueno, K. L.; Martin, SM. and Buettner, G. R. (2002). Comparing, carotene, vitamin E and nitric oxide as membrane antioxidants. *Biol. Chem.*, 383:671-678.
8. Uboh, F. E.; Ekaidem, I.; Ebong, P. E. and Umoh, I. B. (2009). Hepatoprotective effect

- of Vitamin A against Gasoline vapor toxicity in rats. *Gastroenterol. Res.*, 2:162-167.
9. Geecha, O. M. and Fagan, J. M. (1992). Protective effect of ascorbic acid on the breakdown of proteins exposed to hydrogen peroxide in chicken skeletal muscle. *J. Nutr.*, 12:2089-2093.
  10. Ayo, J. O.; Minka, N. S. and Mamman, M. (2006). Excitability scores of goats administered ascorbic acid and transported during hot-dry conditions. *J. Vet. Sci.*, 7(2):127-131.
  11. Suteu, R.; Altuntas, I.; Buyukvanli, B.; Akturk, O.; Koylu, H. and Delibas, N. (2007). The effects of diazozin on lipid peroxidation and antioxidant enzymes in rats erythrocytes: Role of vitamins E and C. *Toxicol. Ind. Health*, 23(1):13-17.
  12. Belge, F.; Cinar, A. and Selcuk, M. (2003). Effects of stress produced by adrenocorticotropin on lipid peroxidation and some antioxidants in vitamin C treated and non-treated chickens. *South African J. Anim. Sci.*, 33(3):201-205.
  13. Urban-Chmiel, R.; Kankofer, M.; Wernicki, A. and Puchalski, A. (2009). The influence of different doses of  $\alpha$ -tocopherol and ascorbic acid on selected oxidative stress parameters in *in vitro* culture of leucocytes isolated from transported calves. *Livestock Sci.*, 127(3): 365-370.
  14. Al-rawi, S. Th. (2014). Environmental impact of nitrate on some physiological and productive traits in dairy cattle. Ph. D., College of Veterinary Medicine, University of Baghdad, Iraq.
  15. Jassim, H. M. and Hassan, A. A. (2011). Changes in some blood parameters in lactating female rats and their pups exposed to lead: effects of vitamins C and E. *Iraqi J. Vet. Sci.*, 25(1):1-7.
  16. Tietz, N. W. (1999). Textbook of clinical chemistry, 3<sup>rd</sup> Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders, Pp: 809-856.
  17. Snedecor, G. W. and Cochran, W.G. (1980). Statistical Methods. 7<sup>th</sup> Ed. The Iowa State University Press American, Pp:476.
  18. Fewtrell, L. (2004). Drinking water nitrate, methemoglobinemia and global burden of disease. *Environ. Health Perspect.*, 112:1371-1374.
  19. Azeez, O. H.; Mahmood, M. B. and Hassan, J. S. (2011). Effect of nitrate poisoning on some biochemical parameters in rats. *Iraqi J. Vet. Sci.*, 25(2):47-50.
  20. Zraly, Z.; Bendova, J.; Svecova, D.; Faldikova, L.; Veznik, Z. and Zajicova, A. (1997). Effects of oral intake of nitrates on reproductive functions of bulls. *Vet. Med. Czech.*, 42:345-354.
  21. Chatterjea, M. N. and Shinde, R. (2005). Textbook of Medical Biochemistry. 6<sup>th</sup> Ed. Jaypee Brothers. India. Pp: 511-513.
  22. Halliwell, B. and Gutteridge, J. C. (1984). Oxygen-toxicity, oxygen radicals, transition-metals and disease. *Biochem. J.*, 219(1):1-14.
  23. Bogard, L.; Bonsignore, J. and Carvalho, A. (1986). Massive hemolysis following inhalation of volatile nitrite. *Am. J. Hemat.*, 22:327-329.
  24. Nakano, T. and Tomlinson, N. (1967). Catecholamine and carbohydrate concentrations in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance", *J. Fish Res. Bd. Can.*, 24:1701-1715.
  25. Sachar, A. and Raina, S. (2014). Effect of Inorganic Pollutant (nitrate) On Biochemical Parameters of the Fish, *Aspidoparia Morar*, *Inter. J. Innov. Res. Sc., Eng. Tech.*, 3(5): 12568- 12573.
  26. Sharma, R. and Kantwa, S. M. (2011). Effects of Vitamin C on Lead Induced Developing Thymus in Mice: A review. *Un. J. Envir. Res. Tech.*, 1(2):91-102.
  27. Rawat, S. K.; Singh, R. K.; Bansode, F. W. Singh, P. and Rana, P. (2013). Potassium nitrate induced toxicity on some hematological parameters of Charles foster rats *J. Recent Adva. appl. Sci.*, (JRAAS), 28:35-38.
  28. Pfeifer, K. F. and Weber, L. J. (1979). The effect of carbon tetrachloride on the total protein concentration of rainbow trout *Salmo gairdneri*. *Com. Bioch. Physiol.*, 64:37-42.
  29. Yousef, M. I.; El-Demerdash, F. M. and Radwan, F. E. (2008). Sodium nitrate induced biochemical perturbations in rats: Ameliorating effect of curcumin. *Food Chem. Toxicol.*, 46:3506-3511.
  30. Helal, E.; Zahkok, S.; Ghada, Z.; Soliman, A.; Al-Kassas, M. and Abdel Wahed, H. (2008). Biochemical studies on the effect of sodium nitrite and/or glutathione treatment on male rats. *The Egyptian J. Hosp. Med.*, 30:25-38.

## تأثير نترات الصوديوم وفيتامين ج على بعض الصفات الكيميوحيوية في الأرناب

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## الخلاصة

صممت هذه الدراسة لمعرفة تأثير إضافة نترات البوتاسيوم وفيتامين ج في غذاء الأرناب على بعض المعايير الكيميوحيوية. استعملت ثمان وعشرون ارنابا نيوزلنديا حيث قسمت إلى أربع مجاميع عشوائياً لكل مجموعة سبع أرناب، غذيت هذه الأرناب على نترات البوتاسيوم وفيتامين ج لمدة 16 أسبوع وكما يأتي: المجموعة الأولى (G<sub>1</sub>) غذيت بنترات البوتاسيوم (168 ملغم/كغم من وزن الجسم/ يومياً)، المجموعة الثانية (G<sub>2</sub>) غذيت بنترات البوتاسيوم (168 ملغم/كغم من وزن الجسم/ يومياً) وفيتامين ج (50 ملغم/كغم من وزن الجسم/ يومياً)، المجموعة الثالثة (G<sub>3</sub>) غذيت بنترات البوتاسيوم (168 ملغم/كغم من وزن الجسم/ يومياً) وفيتامين ج (100 ملغم/كغم من وزن الجسم/ يومياً) و المجموعة الرابعة (G<sub>4</sub>) غذيت على العليقة الأساسية واعتبرت مجموعة سيطرة. أظهرت النتائج أن هناك تفوق معنوي ( $P < 0.05$ ) في كل من الكولسترول، والشحوم الثلاثية واليوريا في المجموعة التي غذيت على نترات البوتاسيوم (G<sub>1</sub>) مقارنة بمجموعة السيطرة (G<sub>4</sub>) في حين أظهرت المجموعتان اللتان غذيتا بفيتامين ج مع نترات البوتاسيوم (G<sub>2</sub>) (G<sub>3</sub>) انخفاض طفيف مقارنة مع المجموعة التي غذيت بالنترات لوحده (G<sub>1</sub>). يستنتج من ذلك أن تغذية الارانب بنترات البوتاسيوم أدى إلى ارتفاع في تركيز الكولسترول، والشحوم الثلاثية واليوريا في المصل، في حين قلل فيتامين ج هذا التأثير.

الكلمات المفتاحية: نترات البوتاسيوم، فيتامين ج، أرناب، الصفات الكيميوحيوية.