LONG-TERM IMPACTS OF NITRATE IN FEED OF RABBITS ON THE HEMATOLOGICAL PARAMETERS AND AMELIORATE ITS EFFECT BY USING VITAMIN C

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Abstract

This study conducted to investigate the effect of nitrate and vitamin C infeed of the females rabbits on the haematological parameters. Twenty-eight adult New-Zealand females rabbits were divided randomly into four groups of 7 rabbits each, were fed nitrate and vitamin C 16 weeks as follow; Group 1 (G1) fed nitrate (170 mg/ kg B.W./ daily), Group 2 (G2) fed nitrate (170 mg/ kg B.W./ daily) and vitamin C (50 mg / kg B.W./ daily), Group 3 (G3): fed nitrate (170 mg/ kg B.W./ daily) and vitamin C (100 mg / kg B.W./ daily) and Group 4 (G4) fed a basal ration as control group. The results showed a significant decreased (P<0.05) in hematological parameters (WBC, RBC, PCV and Hb) in the group that fed nitrate (G1) compared with control group and groups that supplied vitamin C (G4, G2 and G3), whereas the groups that fed vitamin C showed a significant decreased (P<0.05) compared with the control group (G4). From results concluded, the nitrate has inhibition impact on the hematological parameters, while vitamin C decrease this effect.

Keywords: Nitrate, feed, rabbit, vitamin C, hematology.

1. Introduction

The nitrate levels in water and food supplies have been increased during last decades worldwide so far the nitrate pollution has become a global concern which may affect the food quality for daily use and impair the human and animal health [1]. Several studies reported that excessive nitrate intake is a cause many problems, such as methaemoglobinaemia[2]. [3] Recorded that other possible outcomes of prolonged nitrate exposure include cancers, via the bacterial production of N-nitroso compounds, hypertension, increased infant mortality, birth defects of the central nervous system, spontaneous abortion, growth retardation, diabetes, respiratory tract infections, and changes to the immune system. Sharma ,[4] reported the administration of nitrate in drinking water in rabbit caused decreased of WBC, lymphocytes, MCHC, RBC, Hb and basophils. Moreover, nitrate toxicity also causes an elevation in glucose, cholesterol, aspartate transaminase and alanine transaminase in serum rats and rabbits [5][6][7]. The using antioxidant vitamins, such as vitamins A, E and C, to enhance protection against chemical agent-induced toxicity have been widely reported [8][6]. Vitamin C is known to be one of the potent antioxidants that are capable of protecting various biological systems against the toxicity effects of reactive species generated by different chemical agents [9][10], and shown to scavenge free radicals in the body [11].

2. Material and methods

Twenty-eight adult New-Zealand females’ rabbit. Their age were 10-14 months and weight 1800-2200 grams. These rabbits were kept under suitable environment conditions of 20-25°C with lighting program of 12 hours light and 12 hours dark, throughout the experiments, each group was housed in a cage (2x3 meters) containing hard-wood chips as bedding. Rabbits were fed on special formula feed pellets.
Experimental design

Twenty-eight adult female rabbits were divided randomly into four groups, each group consisting of 7 females:

- Group 1 (G1): Rabbits of this group were fed ration which supplemented with nitrate (170 mg/kg B.W. / daily) as a nitrate treated group.
- Group 2 (G2): Rabbits of this group were fed ration which supplemented with nitrate (170 mg/kg B.W. / daily) and vitamin C (50 mg/kg B.W.).
- Group 3 (G3): Rabbits of this group were fed ration which supplemented with nitrate (170 mg/kg B.W. / daily) and vitamin C (100 mg/kg B.W.).
- Group 4 (G4): Rabbits of this group were fed basal ration as a control group.

Blood samples were collected from the adult rabbits from, the blood were collected at zero, 4th, 8th, 12th and 16th week respectively by using anticoagulant tubes to assay the hematological parameters (RBC, Hb, PCV and WBC) by using hemolyzer.

Statistical Analysis:

The data obtained were expressed as means and standard Error (SE) and subjected to statistical analysis using one-way analysis of variance (ANOVA). Also post hoc test was used when appropriate to find the least significant differences (LSD) between groups when ANOVA was statistically significant (P value <0.05 was considered statistically significant) [12]. All statistical analyses were done using SPSS version 20 packages.

3. Results

A total blood samples were collected along five times during the experimental study to evaluate the hematological parameters (WBC, RBC, Hb and PCV). The WBC count are presented in Table 1, the WBCs count were decreased in groups that fed nitrate (G1), the statistical analysis showed a significant increase (P<0.05) in G1, G3 and G4 compared with G1, these increases were observed on eighth, twelfth and sixteenth week respectively.

The mean value of RBCs count were decreased in groups G1 and G2 with the progress of the experiment. A statistical analysis of the results showed a significant differences (P<0.05) between groups G2xG1 at the eighth week. In addition, a significant increase (P<0.05) in group G3 compared with G1 and G2 at the twelfth week. The results also showed a significant increased (P<0.05) of the control group (G4) and the groups that fed nitrate plus vitamin C (G3 and G2) compared with the group that fed nitrate alone (G1) (Table 2).

The concentration of the hemoglobin of the control group G4 recorded a highest value compared with the treated groups G1, G2 and G3 while the lower concentration of hemoglobin was recorded in the group that fed nitrate G1. The results also showed a significant increase (P<0.05) during the eighth and twelfth weeks between groups G4, G3 and G2 compare with nitrate group G1. As well as a significant increase (P<0.05) at the sixteenth week between G4 compare with G2 and G1, in addition a significant increase (P<0.05) between G3 and G2 compare with G1 (Table 3).

The results showed a decreased in the PCV of group one G1 which fed nitrate (170 mg/ kg B.W./ daily) gradually from 38.10±0.83% at zero time until 32.52±1.28% at end of the experiment. A significant increase (P<0.05) were showed on the eighth and twelfth week between G4 compare with G3, G2 and G1. While the groups G4, G3 and G2 showed a significant increase (P<0.05) compared with group G1 at the sixth week (Table 4).
Table 1: The effect of supplementation of nitrate and vitamin C as feed additive to the feed of rabbits on the white blood cells count (X 10³/ul) during the study period

| Groups Time (week) | G₁     | G₂     | G₃     | G₄     |
|-------------------|--------|--------|--------|--------|
| Zero              | 9.04±0.12 | 9.10±0.09 | 9.22±0.23 | 9.12±0.22 |
| 4th               | 7.95±0.14 B | 9.43±0.24 A | 9.15±0.23 AB | 9.42±0.26 A |
| 8th               | 7.73±0.28 B | 9.32±0.27 A | 9.79±0.40 A | 9.95±0.27 A |
| 12th              | 7.82±0.13 B | 9.57±0.23 A | 9.63±0.28 A | 10.08±0.21 A |
| 16th              | 7.70±0.37 B | 9.58±0.41 A | 9.55±0.08 A | 10.11±0.32 A |

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

Table 2: The effect of supplementation of nitrate and vitamin C as feed additive to the feed of rabbits on the red blood corpuscles count (X 10⁶/ul) during the study period

| Groups Time (week) | G₁     | G₂     | G₃     | G₄     |
|-------------------|--------|--------|--------|--------|
| Zero              | 6.64±0.16 A | 6.16±0.04 A | 6.58±0.06 A | 6.27±0.12 A |
| 4th               | 6.55±0.11 A | 6.04±0.10 A | 6.25±0.07 A | 6.47±0.24 A |
| 8th               | 5.80±0.12 B | 5.83±0.09 AB | 6.34±0.14 AB | 6.49±0.14 A |
| 12th              | 5.47±0.12 B | 5.70±0.07 B | 6.27±0.17 AB | 6.85±0.21 A |
| 16th              | 5.01±0.08 B | 5.95±0.18 A | 6.12±0.23 A | 6.47±0.19 A |

The different capital letters refer to significant differences between different groups at (P<0.05).
Table 3: The effect of supplementation of nitrate and vitamin C as feed additive to the feed of rabbits on the hemoglobin concentration (g/l) during the study period

| Groups | Time (week) | G1       | G2       | G3       | G4       |
|--------|-------------|----------|----------|----------|----------|
| Zero   | 126.0±0.41  | A        | 126.50±0.9 | A        | 125.25±2.8 | A        |
| 4th    | 124.0±0.8   | A        | 126.25±0.9 | A        | 126.50±3.25 | A        |
| 8th    | 117.75±2.17 | B        | 126.50±1.5 | A        | 128.50±3.7 | A        |
| 12th   | 112.0±0.71  | B        | 127.60±2.25 | A        | 129.50±4.25 | A        |
| 16th   | 103.75±4.52 | C        | 126.25±1.8 | B        | 129.25±4.75 | A        |

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

Table 4: The effect of supplementation of nitrate and vitamin C as feed additive to the feed of rabbits on the percentage of packed cell volume (PCV %) during the study period

| Groups | Time (week) | G1       | G2       | G3       | G4       |
|--------|-------------|----------|----------|----------|----------|
| Zero   | 38.10±0.83  | A        | 37.52±0.71 | A        | 38.70±3.05 | A        |
| 4th    | 37.91±0.80  | A        | 37.59±0.90 | A        | 39.87±2.45 | A        |
| 8th    | 36.80±0.62  | B        | 36.73±1.92 | B        | 40.03±1.67 | B        |
| 12th   | 35.37±0.79  | B        | 36.52±0.57 | B        | 40.04±2.02 | B        |
| 16th   | 32.52±1.28  | B        | 37.33±0.80 | A        | 39.90±2.33 | A        |

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

4. Discussion

The reduced in white blood cells count was very obvious in groups that fed nitrate, this was due to nitrate as oxidative agents [13][14], which caused damage of white blood cell (WBC) and decreased hematopoietic process to produce new WBC [15], this result is in agreement with Sharma [4]. The protective role of vitamin C on the groups that were given two levels 50 and 100 mg /kg B.W., these levels protected the rabbits and maintain the white blood cells count, vitamin C is effective antioxidant [16][17]. Due to its antioxidant properties and collagen synthesis role it contributes in the regulation of immune system and protection of WBC[18], however, van [19] reported that the oral intake of vitamin C improve the immune system components such as, leucocytes, lymphocyte and T-cell function.

Erythrocytes are particularly sensitive to oxidative damage due to the presence of high polyunsaturated fatty acids content in their membranes and high cellular concentrations of oxygen and hemoglobin[20]. In the present study (Table 3), the groups that fed nitrate showed decreased in the red blood corpuscles count, oxidative damage might be a relevant cause of the initial decrease in the red blood corpuscles count which may be attributed to lysis or shrinkage of erythrocytes in the blood [21]. This result is in agreement with those obtained by [22][23]Mahboob and Bassuny. Rabbits that fed vitamin C showed recovery from the nitrate toxicity, whereas a 100 mg/kg B.W. level was more
effective compared to the 50 mg/kg B.W. level. Vitamin C is an antioxidant, protecting the RBC from hemolysis induced through lipid peroxidation, and it has a protecting effect on erythrocyte membranes exposed to an external oxidative stress [24]. On the other hand, the effects of vitamin C on blood parameters and anemia may result by increasing the iron absorption in the gastrointestinal tract and enhances iron bioavailability [25].

Hemoglobin is a major source for free radicals in red blood corpuscles. It is a reactive molecule, capable of gaining and losing electrons, giving rise to reactive species with the formation of methemoglobin [26] and consequent cell oxidative stress. The results (Table 4) revealed that the administration of nitrate to female rabbits caused a reduction in the hemoglobin concentration in the blood. This change in the hemoglobin concentration as compared to the control rabbits could be explained by, an increase in the activity of the endothelial heme oxygenase by nitric oxide, which degrades heme to carbon monoxide and biliverdin [27]. This result is in agreement with [28] Gluhcheva who suggested that the administration of nitrate caused a reduction in the red blood corpuscles count and decreased a concentration of the hemoglobin.

The improvement caused by using vitamin C may be due to stimulation of the immune system, which results in an increased corticosterone concentration [29], and reduced endogenous formation of N-nitrosodimethylamine and N-nitrosopiperdine from nitrate [30]. In addition, vitamin C can directly reduce methemoglobin and is proved to treat cyanosis effectively [31]. This result is in agreement with [32] Hemza, who found that the administration of sodium nitrate 170 mg/kg B.W. daily caused a decrease of hemoglobin concentration significantly (P<0.05) compared to the control group in rabbits. Similarly, a significant decrease (P<0.05) in hemoglobin concentration and red blood corpuscles count is reported by [33] Baky after administration nitrate. The addition vitamin C (50 mg/kg B.W. and 100 mg/kg B.W.) to the rabbits ration shown an increase of hemoglobin concentration and decreased nitrate toxicity. Vitamin C is an important regulator of iron uptake, it reduced ferric Fe³⁺ to ferrous Fe²⁺ ions, thus promoting dietary non-haem iron absorption from the gastrointestinal tract, and stabilizes iron-binding proteins [34]. These results are agreement with [32] Hemza, who found that the administration of vitamin C (100 mg/kg B.W. daily) to rabbits showed a significant increase (P<0.05) of hemoglobin concentration compared to rabbit that treated with nitrate 170 mg/kg B.W./daily.

The percent of packed cell volume showed the same direction as hemoglobin concentration during the study period, there was a significant decrease (P<0.05) in PCV of a group that fed nitrate (170 mg/kg B.W./daily). Similarly, a significant decrease (P<0.05) in hemoglobin concentration, PCV and RBC count is reported [35]. Groups that were fed vitamin C (50 mg/kg B.W. and 100 mg/kg B.W. daily respectively) showed recovery from nitrate toxicity. The vitamin C act as anti-oxidative damage [36] vitamin C has antioxidant potential which was confirmed by [37] Szeto, which promotes its favorable action on the liver in the regulation of hematopoiesis. These results are in agreement with [38] Yarube, who investigated the addition of nitrate which caused a significant toxicity by decreasing erythrocyte osmotic fragility of the treated rats. Separate administration of both vitamin C ameliorated nitrate toxicity by increasing erythrocyte osmotic fragility back to normal in a dose dependent manner [35].

5. Conclusion

From the results concluded, the increased of nitrate concentration in the feed of the rabbit, this caused decrease in the WBC, RBC, Hb and PCV, while vitamin C enhanced these parameters.

6. References

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