

## Effect of long-term onion (*Allium cepa*) feeding on antioxidant enzymes in goat erythrocyte

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### Summary

The effect of long-term onion consumption on red blood cell antioxidant enzymes [glucose-6-phosphate dehydrogenase (G6PD), superoxide dismutase (SOD) and glutathione peroxidase (GPx)], were measured. The relationship of these enzymes with PCV and hemoglobin (Hb) concentrations was also determined. Twelve adult female goats were used for the experiment and randomly assigned to three groups. Animals of group 1 and 2 received diets containing 30% and 60% spring onions (DM basis) for 60 days, respectively. Goats of group 3 served as control and were fed whole alfalfa hay. Blood samples were obtained before feeding onion and every 10 days up to 80 days. In the onion groups, PCV amounts decreased from day 10 and reached the lowest value at day 40. Although onion consumption reduced PCV, the measures were within the normal range for goats. G6PD activity decreased from day 10 and the lowest value was detected at day 40. After day 40, a slow and gradual return toward the baseline values was seen. In the onion groups, SOD showed a negative correlation with PCV and Hb ( $P < 0.01$ ). On the other hand, there was a positive correlation between G6PD and Hb ( $P < 0.01$ ). It seems that up to 60% of onions in diet can be consumed by goats without noticeable clinical anaemia. Moreover, it seems that SOD has a compensatory role in protection of erythrocytes against oxidative stress induced by onion consumption in goats.

**Key words:** Onion, Antioxidant enzymes, Erythrocytes, Goat

### Introduction

Plants of the *Alliaceae* family such as onions are consumed for their putative nutritional and health benefits (Fenwick and Hanley, 1985; Munday *et al.*, 2003). In areas where onions are grown commercially, it is a common practice to use culled onions as a source of feed for livestock. Livestock may be fed cull onions without any significant problems, as long as they are mixed with other vegetable wastes or other feeds (Hutchison, 1977). However, ingestion of a large amount of onions causes poisoning in some animal species, such as cattle (Hutchison, 1977; Verhoeff *et al.*, 1985; Carbery, 1999; Rae, 1999; Van Der Kolk, 2000), sheep (Van Kampen *et al.*, 1970;

Kirk and Bulgin, 1979; Aslani *et al.*, 2005), horses (Thorp and Harshfiel, 1939; Pierce *et al.*, 1972), dogs (Stallbaumer, 1981; Solter and Scott, 1987), cats (Kobayashi, 1981) and geese (Crespo and Chin, 2004). Experimental onion poisoning has been reported in sheep, dogs, and cats (Harvey and Rackear, 1985; Fredrickson *et al.*, 1995; Robertson *et al.*, 1998; Selim *et al.*, 1999; Figuera *et al.*, 2002).

Onions and other plants of the *Allium* genus contain a wide variety of organosulfoxides, particularly alk(en)ylcysteine sulfoxides. Trauma to the plants, such as chewing, converts the sulfoxides to thiosulfinates. After ingestion by humans and animals, thiosulfinates decompose to a complex mixture of compounds in which

monosulfides, disulfides, trisulfides, and tetrasulfides predominate (Munday *et al.*, 2003).

The primary toxicologic mechanism of onion-derived organosulfur compounds is oxidative hemolysis, which is characterized by development of methemoglobin and Heinz bodies in the erythrocytes (Borelli *et al.*, 2009). Erythrocytes containing Heinz bodies are recognized and phagocytosed by macrophages of spleen and, to a lesser extent, liver and bone marrow, resulting in extravascular hemolysis (Harvey, 2008). Intravascular hemolysis may occur because erythrocytes containing Heinz bodies have decreased deformability and can burst when passing through sinusoids or small capillaries. Another mechanism of intravascular hemolysis is direct damage to the erythrocytic plasma membrane, causing cellular lysis (Borelli *et al.*, 2009).

Oxidative hemolysis occurs when the concentration of oxidants in the erythrocyte exceeds the capacity of antioxidant metabolic pathways. Erythrocytes essentially possess various antioxidant enzymes including superoxide dismutase (SOD), catalase, glucose-6-phosphate dehydrogenase (G6PD) and glutathione peroxidase (GPx) to protect cellular constituents from oxidative degradation (Ogawa *et al.*, 1986). Differences in the activities of some antioxidant enzymes might be the cause of species difference in susceptibility of their erythrocytes to oxidative damage induced by onion (Kasai, 1996).

Among livestock, cattle are more susceptible to the onion toxicity than horses, sheep and goats. A study looking at onion feeding in cattle showed that feeding up to 25% cull onions on a dry matter basis resulted in mild decreases in RBCs, hemoglobin (Hb) and packed cell volume (PCV) but did not result in clinical anemia (Lincoln *et al.*, 1992). Sheep can also be maintained on diets containing up to 50% (DM) onion bulbs with no clinical abnormality and onions can be fed safely to sheep with weight gains comparable to those from whole sorghum grain (Fredrickson *et al.*, 1995).

In Iran, more than 1.5 million metric tons of commercial onion bulbs are annually

produced (FAO, 2003). In most areas, crop harvesting of onions is performed at late autumn when there are some restrictions in providing fresh forages for ruminants, particularly for sheep and goats. So, culled onions and post-harvesting remnants of onion tops are occasionally used for sheep and goat feeding. Onion feeding in such situations can lead to development of hemolytic anemia (Aslani *et al.*, 2005). Based on the authors' knowledge, data concerning experimental onion feeding and safety in goats is not available. The present study was undertaken to evaluate the effect of long-term onion consumption on PCV and Hb concentrations in goats. In addition, changes of erythrocyte antioxidant enzymes after long-term onion consumption and their relationship with PCV and Hb concentrations were evaluated.

## Materials and Methods

### Animals and diets

Twelve clinically normal, female, non-lactating and non-pregnant goats weighing 35-40 kg, 2-4-year-old and stemming from local breeds were purchased from a local market for the study. Goats were randomly divided into 3 equal groups and were housed in indoor pens. Prior to the beginning of the experiment, goats were dewormed and fed under the aforementioned condition for 14 days to ensure proper acclimation. The experiment was approved by the Animal Welfare Committee of the School of the Ferdowsi University of Mashhad.

Spring onions were obtained every day from a local bazaar to ensure freshness. Their dry matter, ash, crude protein and crude fibre contents were determined by standard methods of proximate analysis (Cullison and Lowery, 1987). The dry matter, ash, crude protein and crude fibre content of onions used in the present study have been shown in the Table 1. Animals of group 1 and 2 received a diet containing 30% and 60% spring onions (DM basis) for 60 days, respectively. Goats of group 3 served as control and were fed whole alfalfa hay. Fresh water was available all the time.

### Blood sample collection

Blood samples were collected from all

animals by the jugular vein puncture into sterile microtubes with EDTA or heparin as anticoagulant on day 0 and every 10 days until the 80th day (corresponding to the 20th day after cessation of onion feeding). Large gauge needles were used in order to minimize the *in vitro* haemolysis from mechanical injury to erythrocytes. The blood samples containing EDTA and heparin were used for routine hematological analysis and preparation of erythrocyte haemolysate, respectively.

**Table 1: Dry matter, ash, crude protein and crude fibre content of onions used in the present study**

Parameter	Content
Dry matter	7.5%
Ash	20.2%
Crude protein	22.8%
Crude fibre	10.8%

### Routine hematological examination

PCV and Hb concentrations were determined by microhaematocrit and cyanomethaemoglobin methods, respectively (Jain, 1986).

### Preparation of erythrocyte haemolysate

Immediately after collection, heparinized blood samples were centrifuged at 700 g for 15 min at 4°C. The plasma and buffy coats were removed by aspiration. The sediment containing blood cells was washed three times by re-suspending in isotonic phosphate-buffered saline, followed by re-centrifugation and removal of the supernatant fluid and the buffy coats. One volume of the crude red cells was lysed in nine volumes of ice-cold distilled water to prepare a 10% erythrocyte haemolysate (Chaudhuri *et al.*, 2008).

### Antioxidant enzyme activities

The activities of erythrocyte GPx, SOD and G6PD were determined in erythrocyte haemolysate. Hemolysates were stored frozen at -70°C until analysis. GPx activity was measured by commercially available kits (Ransel test kit, Randox Laboratories Ltd., UK). This method is based on that of Paglia and Valentine (1967). GPx catalyses

the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP<sup>+</sup>. The decrease in absorbance at 340 nm is measured. SOD activity was measured by a modified method of iodophenyl nitrophenol phenyl tetrazolium chloride (INT) (Ransod test kit, Randox Laboratories Ltd., UK). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (INT) to form a red dye. The SOD activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the assay conditions. G6PD activity was measured by commercially available kits (G6PD test kit, Randox Laboratories Ltd., UK). This method is based on that of Komberg *et al.* (1955). The enzyme activity was determined by measurement of the rate of absorbance change at 340 nm due to the reduction of NADP<sup>+</sup>.

### Statistical analysis

Statistical analysis was conducted using SPSS for windows (release 16, SPSS Inc, Chicago, Ill) with a p-value of <0.05 as statistically significant. Repeated measure ANOVA was used for comparison of measured factors in trial groups. For parameters with significant interaction of time and group, one way ANOVA with Bonferroni t-test was used for comparison between groups at each sampling time. Pearson's method was used for determination of the correlation between antioxidant enzymes and RBC parameters (PCV and Hb) in the test groups.

### Results

The values (mean ± SE) of Hb, PCV, GPx, SOD and G6PD in different groups (control, 30% and 60%) are presented in Table 2.

Significant reductions in PCV and Hb according to time (P<0.05) and to the dietary

**Table 2: Effect of onion feeding (30 and 60% of the diet) on some haematological parameters and antioxidant enzymes in goats (n = 12). Results are expressed as mean ± SE**

Haematological parameters	Groups (dietary onion content)			P-values		
	0% (group 1)	30% (group 2)	60% (group 3)	Time	Group	Time x group
PCV (%) †	29.85±2.904	30.31±2.515	30.63±2.904	<0.0001	0.982	0.017
Hb (g/L) †	107.2±8.76	104.1±7.59	106.1±8.76	<0.0001	0.962	0.001
GPx (IU/gHb)	10.8±0.797	10.4±797	11.9±0.797	0.017	0.431	0.258
SOD (IU/gHb)	2764±290.4	3004±290.4	3201±290.4	<0.0001	0.586	0.240
G6PD (IU/gHb) ††	2.15±0.066 <sup>a</sup>	1.74±0.066 <sup>b</sup>	1.75±0.066 <sup>b</sup>	<0.0001	0.003	<0.0001

Means within rows lacking a common superscript differ (P<0.05). \* Significant effect of group (P<0.05). † Significant group and time interaction (P<0.05)

regimen (time x group interactions: P<0.05) were observed in goats fed with onions. In addition, significant difference was seen between control and test 2 (60%) groups for Hb at day 30 (P<0.05). Significant changes in G6PD according to time (P<0.05) and to the dietary regimen (time x group interactions: P<0.05) were observed in goats fed with onions. In addition, G6PD was significantly decreased in goats receiving 60% and 30% onions in diet compared to the control group. Although the SOD and GPx activities have significantly varied according to the time (P<0.05), no significant effect of the group and no significant time x group interaction were evidenced for these parameters.

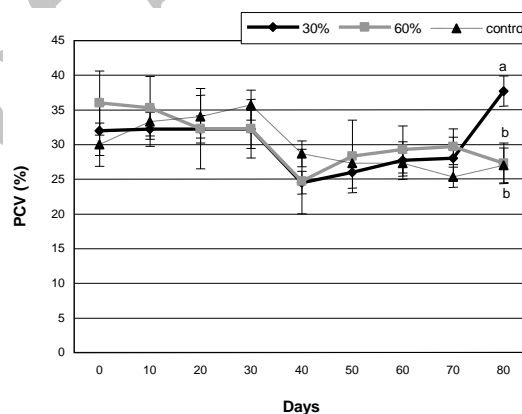
During the experiment, PCV and Hb showed a quite similar pattern of changes in all groups and decreased slowly during the first 40 days. The lowest values were observed at day 40, which were approximately 70% of the baseline values. After that, a slow and gradual increase of PCV and Hb was observed (Figs. 1 and 2). About 1 to 2% of red blood cells showed polychromasia in goats receiving 30 and 60% spring onions at days 50 and 60.

GPx activity changed irregularly (Fig. 3) and no significant difference was observed between groups. The activity of SOD increased during the first weeks of onion feeding and reached approximately 140% of its baseline value at day 40, followed by a slow and gradual decline toward the normal (Fig. 4). No significant difference was seen for the SOD activity between groups.

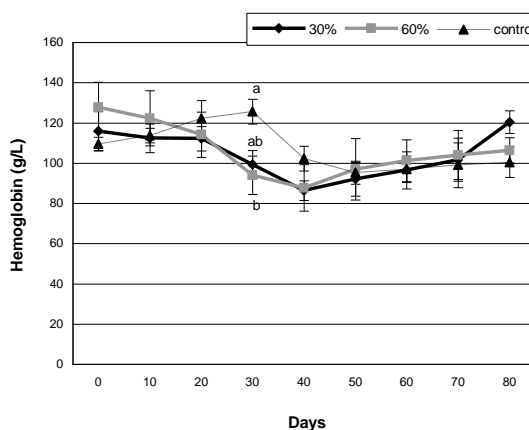
During the first 40 days, G6PD activity of red blood cells in the onion groups decreased about 40 and 50% of the baseline values, respectively. These changes were followed by a gradual rise and return to the baseline values at day 80 of experiment (Fig.

5). G6PD was significantly decreased in goats receiving 60% and 30% onions in diet compared to the control group (P<0.05). Although, the G6PD and SOD activities were changed following long-term onion consumption, these changes were dose independent and there was no significant difference between 30% and 60% groups.

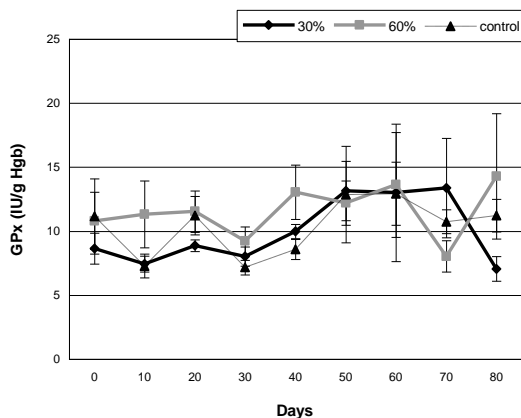
In the 30% and 60% onion groups, SOD activity showed significant negative



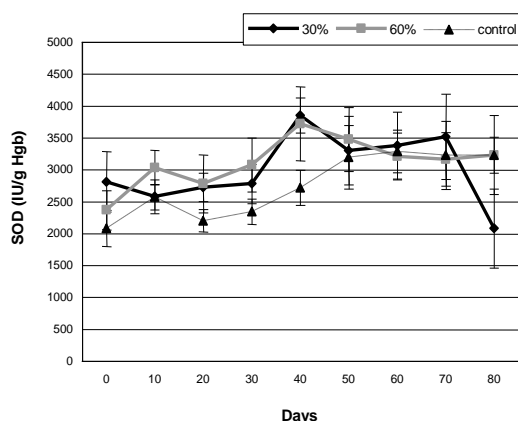
**Fig. 1: Changes of PCV in the goats given 30% and 60% onion in diet. Means lacking a common superscript differ (P<0.05)**



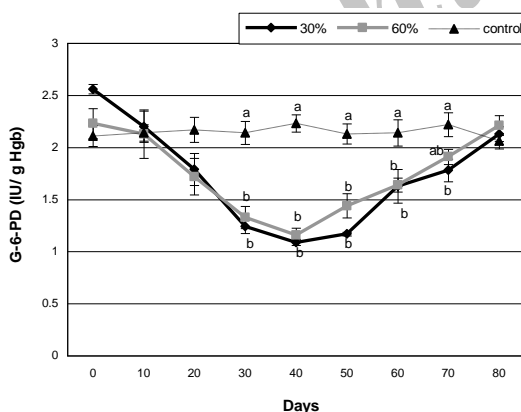
**Fig. 2: Changes of Hb in the goats given 30% and 60% onion in diet. Means lacking a common superscript differ (P<0.05)**



**Fig. 3: Changes of GPx activity in the goats given 30% and 60% onion in diet**



**Fig. 4: Changes of SOD activity in the goats given 30% and 60% onion in diet**



**Fig. 5: Changes of G6PD activity in the goats given 30% and 60% onion in diet. Means lacking a common superscript differ ( $P < 0.05$ )**

correlation ( $P < 0.01$ ) with PCV ( $r = -0.967$  and  $r = -0.851$  in 30% and 60% onion groups, respectively), and Hb ( $r = -0.876$  and  $r = -0.785$  in the 30% and 60% onion groups, respectively). Positive correlation ( $P < 0.01$ ) was seen between G6PD activity and Hb

concentration ( $r = 0.881$  and  $r = 0.849$  in 30% and 60% onion groups, respectively). The correlation tended to be significant ( $P = 0.062$  and  $P = 0.054$ ) between G6PD activity and PCV value ( $r = 0.643$  and  $r = 0.658$  in 30% and 60% onion groups, respectively). There was no significant correlation between GPx activity and PCV and Hb values in the 30% and 60% onion groups.

## Discussion

A notable feature of onion-induced hemolysis is its species selectivity. This may be attributable to the differences in antioxidant defense of the erythrocytes against oxidative stress. Catalase activity, for example, is low in dog erythrocytes (Cope, 2005).

The current study is the first study in which the effect of long-term onion consumption on antioxidant enzymes was studied in goats. In this study, decreased G6PD activities were observed during the first 40 days of onion ingestion. PCV significantly dropped in this period. After day 40 and especially after onion withdrawal at day 60, a slow and gradual return toward the pre-experiment values in the mentioned parameters was seen.

In the current study, goats fed with spring onions showed some degree of hemolysis, evidenced by slight reduction in PCV. The hemolysis in goats fed with 60% onions in diet was evident from day 20, whereas in goats fed with 30% onions in diet, the haemolysis was slightly delayed at day 30. However, in both groups, the erythrocyte destruction appeared maximal at day 40. The erythrocyte parameters increased at the end of experiment (day 80) in goats fed with 30% onions in diet for 60 days, suggesting that erythropoiesis may be markedly stimulated by a slight haemolysis. This resulted in a significant difference between PCV concentration in the 30% group on one hand and the 60% and control groups on the other. Although, onion consumption reduced PCV (Fig. 1), the measures were within the normal range for goats (Kramer, 2000). These results show that goat could stand diets containing up to 60% onions without observing clinical anemia. Similarly, Fredrickson *et al.* (1995)

reported that after 6 weeks of onion consumption (50% DM) by sheep, with the exception of two sheep (from the 14 sheep used in this group), the PCV values in all animals were within the normal range. In addition, it has been indicated that clinical anemia was not observed in cattle feeding diets containing up to 25% onions (Lincoln *et al.*, 1992).

One of the organosulfur compounds of onion, n-propyl disulfide, causes a marked decrease in the activity of G6PD (Tang *et al.*, 2007). This effect was demonstrated in the present study where G6PD activity was reduced following onion consumption and in the study performed by Ogawa *et al.* (1986) in dogs where G6PD showed a transient decrease by 45% of baseline value. The lowest activity of G6PD was observed at day 40, followed by a gradual rise and return to the baseline value. Because the activity of this antioxidant enzyme depends on the age of red blood cells (Glass and Gershon, 1984), its increase after return of RBC parameters toward normal would be evidence of regenerated young cells in the blood circulation (Ogawa *et al.*, 1986). In addition, the positive correlation of G6PD activity with Hb and PCV values showed the importance of this enzyme in the resistance of red blood cells against oxidative damage. The pentose phosphate pathway has a critical significance in the survival of erythrocytes, though only 5% to 13% of glucose is metabolized via this pathway (Harvey, 2008). During this pathway, G6PD catalyzes oxidation of G6P and reducing potential in the form of NADPH is generated in the cell, which helps in maintaining glutathione in the reduced state (Grewal *et al.*, 2005; Harvey, 2008). When the activity of G6PD is decreased, the content of GSH also falls, leading to increased levels of H<sub>2</sub>O<sub>2</sub> (Tang *et al.*, 2007). Thus, G6PD serves as an antioxidant enzyme and decreased activity of G6PD has been associated with increased oxidative stress in endothelial cells (Leopold *et al.*, 2003).

The negative correlation between PCV and Hb concentrations and SOD activity suggested that increased SOD activity is a compensatory mechanism against oxidative damage of red blood cells. SOD catalyzes the dismutation of O<sub>2</sub><sup>-</sup> to H<sub>2</sub>O<sub>2</sub> and has a

central role in the defense against oxidative stress (Beyer *et al.*, 1991; Edwards and Fuller, 1996). Ogawa *et al.* (1986) reported a significant decrease in the SOD activity in dogs with anemia caused by onion feeding. The decreased SOD activity in the anemic dogs might be a consequence of enhanced ROS production and utilization of the antioxidant enzyme to counter the ROS. However, anemia was not observed in the studied goats and ROS production was not high enough to over-utilize this enzyme.

Changes in the activity of GPx were not regular and no significant difference was seen for the GPx activity between groups (P>0.05). These results are in accordance with the findings of Ogawa *et al.* (1986) and Tang *et al.* (2007). Hafeman *et al.* (1974) proposed that GPx plays a crucial role in preventing membranes from peroxide damage induced by lipid peroxides. GPx catalyzes the conversion of H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O. This reaction is important because the accumulation of H<sub>2</sub>O<sub>2</sub> might decrease the lifespan of erythrocytes by increasing the rate of oxidation of hemoglobin to methemoglobin (Winterbourn, 1985). Regarding the role of GPx in preventing membranes from lipid peroxidation, it seems that onion feeding in goats does not produce lipid peroxides high enough to alter GPx synthesis and activity.

It seems that up to 60% spring onions in diet can be consumed safely by goats without any clinical anaemia. G6PD activity in the goat erythrocytes was decreased after onion consumption. The negative correlation between RBC parameters and SOD activity suggested that SOD plays a compensatory role in protection of erythrocytes against oxidative stress induced by onion consumption in goats.

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