

## **Effect of vitamin C on haematology and serum biochemistry in heat-stressed sheep**

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### **Abstract**

The present study was carried out to determine the effect of different doses of vitamin C on some blood parameters and serum biochemistry in heat-stressed sheep. A total of sixteen ewes were divided into four groups. First group was kept as control. Other three groups were treated orally with three doses of vitamin C (25, 50 and 75 mg/kg/day) for 30 days. At the end of the experiment, the results showed significant increase in blood parameters in the vitamin C supplemented groups including red blood cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), lymphocyte and monocyte, while white blood cells (WBC), neutrophils and eosinophils decreased in treated groups. In serum biochemical parameters including glucose, cholesterol, total protein and glutamic oxaloacetic transaminase (GOT) concentration increased in treated groups.

**Key words:** Vitamin C, Heat Stress, Sheep

### **Introduction**

High ambient temperature induces series of drastic changes in sheep biological function that disturb metabolism of water, protein, energy and mineral balance, enzymatic reaction hormonal secretion and blood parameter (Habeed et al., 1992). Vitamin C acts as a highly effective antioxidant in the body, where it protects it from damage by free radical and reactive oxygen species that can be generated during normal metabolism as well as through exposure to stress. Vitamin C may also be able to regenerate other antioxidants such as vitamin E (Bruno et al., 2006). Vitamin C is also important for immune system and increases production of antibodies, prostaglandin, T-lymphocytes and interferon (Eberhard et al., 1989). The role of vitamin C is needed for the synthesis of collagen, necessary for blood vessels structure, tendons ligaments and bones. More recent research also suggests that vitamin C is involved in the metabolism of cholesterol to bile acids and the formation of adrenal hormones which may have implication for blood cholesterol levels (Carr and Frei, 1999). Due to wide range applications of vitamin C, a study was design to find different doses of this antioxidant on haematology

and serum biochemical parameters in sheep subjected to summer stress in Iraq.

### **Materials and Methods**

The trial carried out in animal's field of Agriculture Collage of Basrah University, Iraq. The experiment was started in summer season when average temperature ranged from 38 to 45°C. Sixteen female sheep were divided into four groups consisted of four ewes each: Group one: treated with distil water orally and considers as control.

Group two: treated with vitamin C at doses 25 mg/kg BW/daily orally.

Group three: treated with vitamin C at doses 50 mg/kg BW/daily orally.

Group four: treated with vitamin C at doses 75 mg/kg BW/daily orally.

In the present study, vitamin C was used as L-ascorbic acid (Bayer Pharmaceuticals, France). The vitamin C was dissolved adequately and drenched to each sheep in the morning for 30 days. At the end of the experiment, 5 ml blood was taken from each animal into 2 test tubes (one with EDTA and the other without EDTA). Blood with EDTA was used to measure RBC

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and WBC count with the help of haemocytometer (Neubauer improved double) using the method of Sood (1966) and Dacie and Lewis (1984) respectively. The concentration of Hb and PCV were measured according to the method of Schalm et al. (1975).

Blood without EDTA was centrifuge at 1500 rpm for 20 minutes to separate serum. Serum cholesterol and glucose and total protein were measured by using chemical kit SPECTRUM (Egyptian Company for Biotechnology). The GOT was measured using a chemical kit (BIOMÉRIEUX®, France).

Data was analyzed statistically using analysis of variance (SPSS, version 9.00). Where there was significant difference, multiple range test was used (Duncan, 1955).

## Results

The result of RBC, PCV and Hb concentration are shown in Table 1. The animal groups treated with 50 and 75 mg/kg body weight of vitamin C improved significantly ( $P \leq 0.05$ ) the concentration of RBC, PCV and the Hb concentration compared with control group.

The Table 2 shows that vitamin C was able to reduce the white blood cells (WBC) significantly ( $P \leq 0.05$ ) compared with the control. It was also observed that vitamin C has the capability to decrease the percentage of neutrophil (75 mg/kg) and increase the lymphocyte percentage (50 mg/kg) significantly compared with control.

The results in Table 3 indicate the role of vitamin C has significant ( $P \leq 0.05$ ) effect on glucose value compared with control group. Cholesterol, total protein

and GOT had no significant difference between control and treated groups.

## Discussion

Vitamin C improved blood parameter in three doses groups but the most effective were 50 and 75 mg/kg compared with the control animals, indicating that vitamin C protects from oxidative stress as result of exposure of animals to heat stress (high ambient temperature). Vitamin C has many important functions in the body such as synthesis of neurotransmitter formation, norepinephrine from dopamine and critical to function of nervous system. Vitamin C is also required for synthesis of carnitine that was essential for the transport of fat in to cellular mitochondria, where fat is converted to energy (Carr and Frei, 1999).

The elevation of RBC count in the present study may be due to the role of vitamin C on blood formation which has been reported previously. The administration of vitamin C improves the availability of iron found in tissue storage site (NPA-publication 2003). Gaby and Singh (1991) suggested that absorption of iron specially the non hem are enhanced by vitamin C. Vitamin C facilitates iron absorption by its ability to reduce ferric iron to the ferrous form (Sayers et al., 1973), vitamin C acts as antioxidant to protect RBC membranes (consist of phospholipids) against the harmful oxidation processes and thus reduce the R.B.C. haemolysis via increasing the flexibility of the cell membranes (Nguyen et al., 2001). The recent studies have confirmed that vitamin C plays an important role in response to erythropoietic agents (NPA, 2003).

**Table 1: Effect of different doses of vitamin C on blood parameters of sheep exposed to summer heat**

Groups	RBC ( $\times 10^6/\text{ml}$ )	PCV (%)	Hb (mg/dl)
Control	$3.80 \pm 0.300^b$	$27.66 \pm 2.51^b$	$8.20 \pm 0.75^b$
25 mg/kg	$4.33 \pm 0.42^{ab}$	$30.00 \pm 2.6^b$	$8.26 \pm 0.30^b$
50 mg/kg	$4.82 \pm 0.17^a$	$38.00 \pm 2.00^a$	$11.46 \pm 0.32^a$
75 mg/kg	$4.81 \pm 0.31^a$	$37.00 \pm 2.51^a$	$11.13 \pm 0.76^a$

Means with different superscripts in a column differ significantly ( $P \leq 0.05$ )

**Table 2: Effect of different doses of vitamin C on heat-stressed WBCs count in sheep**

Groups	WBC ( $\times 10^3/\text{ml}$ )	Neutrophil %	Eosinophil %	Basophil %	Lymphocyte %	Monocyte %
Control	$8.86 \pm 4.93^a$	$67.66 \pm 2.51^a$	$6.00 \pm 1.00^a$	0	$22.33 \pm 2.51^c$	$4.00 \pm 1.00^b$
25 mg/kg	$6.73 \pm 1.40^{ab}$	$58.33 \pm 2.88^{ab}$	$3.66 \pm 1.52^{ab}$	0	$31.33 \pm 2.30^b$	$8.00 \pm 3.46^a$
50 mg/kg	$5.46 \pm 2.36^b$	$61.66 \pm 5.77^a$	$5.66 \pm 1.52^{ab}$	0	$36.66 \pm 2.88^a$	$4.00 \pm 1.00^b$
75 mg/kg	$6.13 \pm 1.02^{ab}$	$48.33 \pm 10.40^b$	$4.33 \pm 0.57^{ab}$	0	$34.66 \pm 0.57^{ab}$	$4.60 \pm 0.57^{ab}$

Means with different superscripts in a column differ significantly ( $P \leq 0.05$ )

**Table 3: Effect of vitamin C on serum biochemistry of heat-stressed sheep**

Groups	Glucose (mg/dl)	Cholesterol (mg/dl)	Total protein (gm/dl)	GOT (I.U)
Control	$29.81 \pm 1.62^b$	$34.36 \pm 3.52^a$	$3.97 \pm 2.31^a$	$39.33 \pm 4.93^a$
25 mg/kg	$33.00 \pm 1.00^a$	$33.86 \pm 4.21^a$	$5.10 \pm 3.26^a$	$38.33 \pm 5.85^a$
50 mg/kg	$32.00 \pm 1.00^{ab}$	$33.13 \pm 2.21^a$	$5.07 \pm 6.09^a$	$38.00 \pm 6.08^a$
75 mg/kg	$33.33 \pm 2.51^a$	$32.56 \pm 10.40^a$	$5.23 \pm 1.90^a$	$37.66 \pm 4.72^a$

Means with different superscripts in a column differ significantly ( $P \leq 0.05$ )

The haemoglobin concentration also increased due to the role of vitamin C which increases absorption of iron from digestive system and it also acts on the reduction of bivalent copper ion ( $\text{Cu}^{++}$ ) in to monovalent copper ion ( $\text{Cu}^{+}$ ) and thus acts as coenzyme to increase haemoglobin (Harper et. al., 1979). The increased in PCV may be due to the increased in total RBC count (Alghannami, 2004).

Vitamin C assists the immune system in two of its primary function to rid the body of foreign invaders and to monitor the system for any sign of tumour cell. It accomplishes these vital tasks by stimulating the production of white blood cells primarily neutrophils, which attack foreign antigens such as bacteria and viruses. Vitamin C is important for immune system and increases production of prostaglandin, T-lymphocytes other white blood cells (Eberhard et al., 1989). The supplementation of vitamin C has been shown to enhance the plasma ascorbic acid concentration and immune response of the cattle subjected to environment stressors (Padilla et al., 2007).

The present study observed elevation in the glucose and total protein levels. The exposure to heat stress increases glucose level of sheep (Sano et al., 1985). Increase glucose and total protein concentration may be due to reduction of oxidative stress produced by heat stress (Bruno et. al., 2006). Vitamin C increases food consumption and food conversion (Cromwell et al, 1970) and causes an increase in thyroid gland weight thus increases its activity which means increasing the secretion of thyroxin hormones (Al-Katib, 2001). Hoshion et al. (1991) suggested that the thyroid hormones have important role in enhancement and growth of calves due to function of thyroid hormones which increases food consumption, absorption from digestive system and finally metabolic rate. The present study shows a beneficial effect of vitamin C in high doses on the blood picture of heat-stressed sheep.

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