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Study of Vitamin C Role on Certain Blood Component of Confinements Stressed in Local Goats Breeds

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Abstract. This study included the definition of over crowding in traditional settlements, confinement of animals and not going out to graze in natural pastures which causes stress and its impact on the characteristics and standards in the blood. Metabolism for two weeks by oral dosing of 3 g, 6 g and control group and we found that the best use of a concentration of 3 g gives good results for adaptation and changes the number of white blood cells, as there was a significant increase in stressed animals when adding vitamin C and the variation decreased significantly($P < 0.05$) for the lymphocytes and the value of granulocytes increased, significant ($P < 0.05$) differences were found between the values and the number of granulocytes, lymphocytes in a good measure to indicate changes caused by environmental stress, overcrowding and there is a significant increase in the values number of red blood cells and that vitamin C will enhance immunity and the ability to resist and adaptation.

Keywords: ascorbic acid, WBC, LYM GRA, RBC, confinement stress

Introduction

The animal needs to live in habitat, graze in natural pastures. These plants provided the proteins, mineral elements and vitamins but in the event if pastures are not available in critical seasons or areas of dry farming (Jaber *et al*., 2013) and animals are raised in traditional houses which restricts their movement and compete for food and place, forcing the animal to adapt, as a result of this change and over crowding of the dwelling is one of the causes of stress.

Goats have biological properties compared to other ruminants in terms of their ability to withstand environmental conditions.

Prevalence in the region and the lack of crude protein and phosphorous in feed, it has the ability to adapt for living and production, especially in the dry areas (Chilliard *et al*., 2003). It was found that goat breeds in arid and semi arid areas are smaller than European breeds (Daramola *et al*., 2021).

When the animal is exposed to stress, the animal resorts to reducing feed consumption and nutritional deficiency leads to specific diseases such as iron deficiency. The addition of nutritional supplements compensates for the deficiency (Padayatty and Levine, 2016) because stress causes mal absorption of the intestine of nutrients and their loss and leads to weak resistance, the lack of vitamin C in ruminants causes anaemia (Chilliard *et al*., 2003) and ascorbic acid is considered an antioxidant because ascorbate protects the body against stress oxidation and is considered a catalyst in enzymatic reactions, the most important of which are collagen synthesis reactions and metabolism reactions. Vitamin C is self produced by almost all types of living organisms, except for some of them. Ascorbic acid is derived from glucose in plants and most mammals, as it is made in the liver and stored for 3-4 months reported by (Padayatty and Levine, 2016).

In cases of stress, vitamin C is released with stress hormones because it enters the catabolism reactions of the amino acid tyrosine during the manufacture of adrenaline, as the amount of vitamin C that is excreted in urine increases in cases of psychological, emotional and physiological stress (Padayatty and Levine, 2016). To find out the degree of adaptation of the animal, the dummy image is examined as an indicator of the health status Hussain and Salman (2012) as a result of change of environmental conditions and to detect anaemia in local goats.

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Material and Methods

This study was conducted to investigate 1st July, 2021 to 15th August, 2021 is the effect of adding vitamin C at different levels as food additive and its effect on the blood parameters of local black goats.

The experiment used 12 animals aged 1.5-2 years, males weighed 30-35 Kg and adult females weighed 18-24 Kg. The animals were randomly distributed in a semi open barn that does not go out for grazing and the type of dwelling is crowded confinement.

The control group was not given any vitamin C, the second treatment was given 3 g of vitamin C and the third treatment was given 6 g of vitamin C.

It was fed on a standardized diet 2% of the body weight in addition to straw (Kim *et al*., 2019) and drinking water was provided. Vitamin was dosed to the fasting animals in the early morning. The control group was dosed with water only.

Collection of blood samples two weeks after the preparatory period. Blood samples of fasting animals were collected before the experiment. Ten mL was withdrawn from the jugular vein through a ten cubic centimeter medical syringe and placed in test tubes in an ethylene diamine tetra acetic acid (EDTA) container. The samples were transferred to the laboratory and then the blood parameters were estimated with a Complete blood count (CBC) device (Chhabra, 2018).

This device is used to give a complete picture of blood components. The condition of passing clotted masses of blood components. The Coulter device of the CBC operation counts white blood cells, red blood cells and platelets (blood components).

The measurement method depends on the presence of an electrician field. It is saturated with an Isolone solution to equalize the charges, and there is a column through which had constant voltage current passes when one of the components of the blood passes, it generates partial resistance to the electric current passing through the electric rod and the strength of the resistance generated by this component is measured. According to the size of passing blood metrics and the intensity of electrical resistance, these components are distinguished as follows after two weeks of dosing and samples were withdrawn from the jugular vein for all experimental animals to compare the results of the two with drawals parameters. The white blood cells (WBC), lymphocytes (LYM), mid range absolute count (MID), granulocyte neutrophil (GRA), mean corpuscular hemoglobin (MCH), red blood cells (RBC). Before adding the vitamin and after adding T1 = control; T2 = add 3 g; T3 = add 6 g.

Statistical analysis. We used the complete random design program, CRD and tested the significant differences between the means using less than RLSD and using the ready made statistical program SPSS 2008.

The level of significance was scored at $P < 0.05$.

Results and Discussion

Although goats are considered well adapted to the tropical climate, now their response to high temperatures of 38 °C and above, they activate their physiological ability to adapt in terms of changes in behaviour, physiological responses and biochemical responses to the blood and endocrine glands to regulate their body temperature (thermal homeostasis) (Al-Bulushi *et al*., 2017). Analyses of some biological indicators of goats where it was found that the average values of (WBC) count for the experimental treatments before the experiment. Table 1, showed analysis the control group, the second treatment within the normal ranges and the third treatment recorded the highest value and the values were as follows:

$(14.56\pm3.32, 14.50\pm8.08, 17.56\pm5.23) \times 10^3$ cells/mL.

The addition of vitamin C found significant differences between the treatments and Table 2 showed analysis the second and third treatment showed a decrease in the number of white blood cells, while the control group found a slight increase in the number of white blood cells and the third group recorded a clear decrease and the values were as follows:

$(15.42 \pm2.63, 9.04 \pm0.71, 5.91 \pm1.91) \times 10^3$ cells mL.

We noticed that there are differences significant between the T1, T2, T3 that the number of (WBC) decreased in the T3 than in T2 a goats had rang WBCs 8.05-$9.0\times10^3/\mu$L, the reason may be that vitamin C enhances the immune system, helps in the production of (WBC) and is an antioxidant. Recent research has indicated a decrease in the number of Neutrophils in the blood of animals treated with vitamin C, even if the treatment period was short for several days which gives a sustainable effect. The lack of vitamin C leads to decrease in immunity and an increase in the number of immature and inactive neutrophil cells due to the body's need for

it and having a longer life span than healthy cells. Vitamin C protects these cells from programmed death, especially for cells sensitive to oxidation after activating the work of Neutrophils (Akinmoladun *et al*., 2020) and the decrease in the number of white blood cells may be due to another reason. Recent research has proven that animals treated with vitamin C lead to a decrease in the number of monocytes in plasma for the same reason as above (Ang *et al*., 2018).

The values of lymphocytes (LYM), for the T1, T2 and T3 before the vitamin C administration were as follows: (8.07±3.28, 8.68±5.61, 9.35±2.10) µL.

Due to stress, while the values were after dosing. Due to stress the values after dosing were (8.87±1.73, 4.06±0.20, 2.61±0.85) µL where we notice significant difference between the T1, T2, T3 if the number decreased with the T3 from the T2 and the T1. Studies show that stress leads to the activation of bacterial genes in the intestinal microbes from the immune cells and thus the immune cells function Small B and T third line of defense and large natural killer cells NK as an antioxidant and that oxidative stress leads to the accumulation of cells affected by aging, dysfunction and mutations, the reason may be attributed to the effect

Table 1. Before supplement vit. C T1 control, T2: add 3 g, T3: add 6 g (mean ± SD)

Parameters	Treatments			Significant
	T1 ± SD	T2 ± SD	T3 ± SD	
WBC (×10³/µL)	14.56±3.32	14.50±8.08	17.56±5.23	N.S
LYM%	55.30±12.07	57.60±8.30	60.47±7.97	N.S
MID%	21.14±6.01	18.48±3.98	17.60±4.95	N.S
GRA%	23.54±7.25	23.98±7.26	21.90±5.36	N.S
LYM	8.07±3.28	8.68±5.61	9.35±2.10	N.S
MID	2.90±0.62	2.50±1.33	2.70±1.14	N.S
GRA	3.44±1.02	3.32±1.40	3.40±0.56	N.S
HGB	8.10±1.43	7.76±2.20	5.78±2.22	N.S
MCH	3.41±0.49	3.08±0.36	3.17±0.47	N.S
RBC (×10⁶/µL)	2.39±0.48	2.47±0.49	2.17±0.09	N.S
MCV	31.20±0.44	31.00±0.71	31.40±0.89	N.S
PDWs	8.02±0.25	8.24±0.18	7.98±0.27	N.S

a, b and c = means in the same row with different letters show significant differences between treatments (P<0.05); SD = standard deviation; NS = non-significant.

Table 2. After supplement vit. C T1 control, T2: add 3 g, T3: add 6 g (mean ± SD)

Parameters	Treatments			Significant
	T1 ± SD	T2 ± SD	T3 ± SD	
WBC(×10³/µL)	15.42a±2.63	9.04b±0.71	5.91c±1.91	*
LYM%	57.36a±2.41	45.22b±4.33	44.26b±3.69	*
MID%	11.30b±1.40	12.88b±2.27	16.44a±1.73	*
GRA%	40.74b±6.64	51.90a±3.44	48.70a±5.69	*
LYM	8.87a±1.73	4.06b±0.20	2.61b±0.85	*
MID	1.78±0.41	1.43±0.57	1.86±1.11	N.S
GRA	3.15c±0.37	5.91a±0.88	4.50b±0.63	*
HGB	9.26a±0.95	7.56ab±1.14	6.20b±2.48	*
MCH	6.64b±0.57	8.82a±0.11	8.38a±0.36	*
RBC(×10⁶/µL)	1.33b±0.46	8.02a±2.92	9.62a±3.15	*
MCV	37.12±1.25	36.56±0.61	37.82±2.11	N.S
PDWs	13.68±0.88	12.86±0.81	12.72±1.30	N.S

a, b and c = means in the same letters show row with different significant differences between treatments (P<0.05); SD = standard deviation; NS = non-significant.

of vitamin C in the cause of the occurrence and regulation of the immune system and the regulation of the work of genes and the function of immune cells in the blood in stress and trying to adapt stress also produces an increase in cholesterol levels in the blood, through the action of adrenaline and nor adrenaline to release free fatty acids, the body contains (2-20) times the number of immune cells and here appears the role of ascorbic in stem cell differentiation, as immune cells undergo dramatic metabolic changes after activation and increase the activity of aerobic glycolysis and oxidation of fatty acids and this leads to altering the phenotype to reach Immune balance (Sara *et al*., 2016), The decrease in lymphocytes can be explained by the decrease in stress as a result of using ascorbic.

MID is the combined value of monocytes, basophils, eosinophils and immature and mature cells. MID values were recorded for T1, T2 and T3, the values were as follows:

(2.90±0.62, 2.50±1.33, 2.70±1.14).

The number may change due to reduced immunity and imbalance of hormonal and anaemia (Gorkhali *et al*., 2017).

After the addition of vitamin C, the values in the blood serum changed by means of dosing were:

(1.78±0.41,1,43±0.57,1,86±1.11) and there were no significant differences between the treatments. The relative stability of monocytes, eosinophils and basophils may indicate the result of the adaptation of the animals to the experimental period.

The results of the blood cells analysis show the rate of number Granulocyte. It's a good indicator to measure physiological changes and has a good measure to know the harmful effect of heat stress for the long time on the immune system. It is characterized by its short life and high degree of movement and represents 40-70% of the total number of white blood cells and in cases of stress, the (GRA) stay for several hours in blood 6-10 then leave to the tissue for 2-3 days and from its kinds neutrophil and its rate in Damascus goats blood 39%, eosinophil's 6%, basophils 1.36% and lymphocytes 47% and monocytes 5% (Al-Bulushi *et al*., 2017).

The number of poly morphonucler leukocytes will increase old and incomplete cells and accumulate on the walls of the arteries may lead to their closure in cases of severe tension (Mohammed *et al*., 2013) and the values of granulocytes before the experiment for the T1, T2, T3 as follows:

(3.44±1.02, 3.32±1.40, 3.40±0.56)p g.

After adding vitamin C, the (GRA) values were as follows:

(5.91±0.88, 4.50b±0.63, 3.15c±0.37)p g.

It was found a significant effect and superiority in the T2 over the T3. The vitamin C had an effect that the neutrophil cell, the rates were almost constant due to the effect of the enzyme L-gulono-γ-lactone oxidase (the active enzyme in vitamin C) which is made in the rumen of ruminants self by micro-organisms and is derived of the glucose in plants (Zhou *et al*., 2018) helps the enzyme GLO, its chemical formula is $C_6H_8O_6$, the active substance oxo-l-thero-hexono-1,4-2 lactone-2,3-enediol. E-demolition of the amino acid tyrosine during the manufacture of the hormone adrenaline and to enhance the work of the immune system and adaptation in cases of stress (Patananan *et al*., 2015) but in cases of stress, it is less manufactured.

That means the values of hemoglobin in red cells were MCH for the T1, T2, T3 as follows:

(3.41±0.049, 3.08±0.036, 3.17±0.047)p g.

It was noted that there were no significant differences. The appearance of abnormal results indicating the presence of anemia or small size of blood cells due to iron deficiency or folic acid deficiency. After adding vitamin C, the results of hemoglobin analysis were as follows:

(6.64±0.057, 8.82±0.11, 8.38±0.036) and we note the superiority of the T2 over the T3. Vitamin C is an effective treatment and contributes to stimulating the formation of hemoglobin to increase resistance and reduce the harmful effects of adapting to unsuitable conditions. The reason may be due to the slowing down of the steroid hormone because there is an inverse relationship between vitamin C and the rate of secretion of the hormone corticosterone in response to the release of the adreno-corticotropic hormone (ACTH) under stress (Zubair *et al*., 2020) and because ascorbic acid is in reducing agent for iron and copper minerals and enhances iron absorption and keeps it in the reduced state (Okoruwa *et al*., 2014).

Red blood cells (RBC) has a vital role and helps in the breathing process contains hemoglobin protein. The

results of the red blood cells examination show the effects of the values a lot. Animals are exposed to stress as a result of environmental stress and their number apparently decreases. The values are due to iron deficiency. Vitamin C and mineral deficiency may be the values of the T1, T2, T3 were as follows:

(2.39±0.48, 2.47±0.49,2.17±0.09) $\times 10^6$ cells/mL. After adding vitamin C, the values were found for the line T1 and T2 were as follows:

(1.33±0.46 ,8.02 ±2.92,9.62±3.15) $\times 10^6$ cells/mL.

The number of red blood cells for the control group is low and the T2 is superior to the T3. It is known that the ability of red cells in mammals to change their shape under a level of pressure without rupture is a cellular property determined by the engineering and physical properties of the cell membrane.

The reason may be the fragility of the corpuscles which are part of the physiological variance and defects that form the membrane (Igbokwe and Igbokwe, 2015) and are affected by genetic factors, genes, diseases, environmental stress (Sejian *et al*., 2018) and over crowding in housing, confinement, thermal and nutritional, results to the lack of cholesterol and phospholipids in the cell membrane.

In the number of red blood cells for the second and third treatment as a result of the effect of ascorbic acid and re-synthesis in the liver and there is a moderation in the clarity of the membranes because ascorbic modifies the composition of the double layer of phospholipids that make up the cell membrane and the effect of ascorbic by dilation and constriction of the arteries and blood flow and helps the absorption of non-heme iron available in plants.

A non-heme iron Ahmad and Ashutosh (2018) being an antioxidant protects cells from deformations as a result of the increase in free radicals caused by stress, due to the increase of hormone Cortisol which results in the decrees of eosinophil's cells and that's for it role in removing venomous protein.

Conclusion

Grazing in natural pastures is considered a luxury for goats and raising in barns causes a kind of tension and depletion of the self supply of vitamin C which affects the health of the animal, Vitamin C is a reducing agent and it is necessary to maintain minerals in a reduced state to resist stress, ascorbate is an ion of ascorbic acid that is essential for basic metabolic reactions and is synthesized by auto ruminants in the presence of the enzyme GLO and participates in the metabolic pathways (HMP) pentose phosphate pathway, the formation of the enzyme GLO is not complete in cases of stress which leads to a decrease in the manufacture of vitamin C and may cause anemia, there are significant differences in the blood parameters of stressed animals when using vitamin C as a food supplement at a concentration of 3 g/animal.

Conflict of Interest. The authors declare that they have no conflict of interest.

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