EFFECT OF AGING ON ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANT STATUS IN SAANEN GOATS

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Abstract. Aging is a series of irreversible structural and functional changes in body molecules, cells, tissues, organs and systems. It is an inevitable process in each organism's life. Free radical theory of aging is one of the most widely accepted theories. This study was conducted to determine the effects of aging on the oxidant-antioxidant status by quantifying malondialdehyde-index of oxidative stress and some antioxidant parameters in erythrocytes of Saanen goats. The effects of aging on erythrocyte oxidant-antioxidant values were investigated on 15 kids agged 3–4 month and 15 mother goats 4–5 years of age. The level of serum malonyldialdehyde (MDA) was determined to be significantly higher in mother goats than kids ($P<0.05$). The levels of glutathione (GSH) and glutathione peroxidase (GSH-Px), the antioxidant parameters, were observed to be higher in mother goats than kids ($P<0.05$) whereas catalase (CAT) enzyme activity was to be lower in mother goats than kids ($P<0.05$). While serum β-carotene concentrations in mother goats were determined to be higher than kids ($P<0.05$), it was identified that there were no important difference in levels of plasma vitamin C (Vit C) and serum ceruloplasmin between kids and mother goat ($P>0.05$). As a result, antioxidant parameters were affected by aging process in Saanen goats.

Keywords: aging, Saanen goat, oxidant, antioxidant

Introduction. Aging is a series of irreversible structural and functional changes in body molecules, cells, tissues, organs and systems. In the course of time, it becomes an inevitable process in each organism's life. By aging body harmony and ability to cope with changing conditions and consisting stresses decrease (Demirol et al. 2006). A lot of theories have been claimed explaining these harmful effects observed during aging process. One of the most important of these theories is free radical theory causing oxidative damage in bodies (Beckman and Ames, 1998). Recently, stress, pollution and consumption of prepared food are thought to increase the production of free radicals in human bodies (Prasad et al. 2002).

Oxidative stress is believed to play an important role in regulating the metabolic activity of some organs and productivity in farm animals. A stressful condition leads to the excessive production of free radicals, which results in oxidative stress, an imbalance in the oxidant/antioxidant system. Generation of free radicals is an integral feature of normal cellular functions. In contrast, excessive generation and/or inadequate removal of free radicals results in destructive and irreversible damage to the cell (Lopaczyski and Zeisel, 2001). Reactive oxygen species (ROS), superoxide radical, hydrogen peroxide and hydroxyl radical have a great impact on the normal function of biomolecules like nucleic acids, proteins and cell membrane phospholipids. Free radicals are generated during stepwise reduction of molecular oxygen (Singh et al. 1999).

The antioxidant system plays a vital role in the protection of cells against oxidative stress in aerobic organisms. Antioxidants protect the cell towards toxic effects of lipid peroxidation by preventing peroxidation chain reaction or collecting reactive oxygen types. Enzymes with important antioxidant functions include catalase (CAT), which catalyses the breakdown of hydrogen peroxide to oxygen and water, and glutathione peroxidase (GSH-Px), which facilitates the destruction of both hydrogen peroxide and organic peroxides. Reduced glutathione (GSH), a tripeptide thiol, is an important antioxidant, as well as a co-factor for various antioxidant enzymes (Kidd 1997). Vitamin C and β-carotene are included in the non-enzymatic parts of antioxidant system. Vitamin C decreases the adverse effects of reactive oxygen and nitrogen species that cause oxidative damage to macromolecules such as lipids, proteins, and DNA. Carotene is a precursor of vitamin A that is a substrate important for the free radical reactions and powerful singlet oxygen scavengers in low oxygen pressures. β-carotene is known to help repair damaged tissue and therefore may be beneficial in counter-acting free-radical damage (Chiu et al. 2008).

In various studies analyzing the oxidant and antioxidant conditions of test animals such as mare, rat and mouse and people that are aging, lipid peroxidation products are reported to be usually high. Even the reason of oxidative stress which increases because of aging is thought to be antioxidant defence system's weakening; changes about aging in parameters have been seen to give incoherent results (Yargıcoglu et al. 2001; Aydilek and Simsek, 2006; Mehdi et al. 2012; Geyikli et al. 2013) The literature scans done by us showed that there are no

studies about the effect of aging on erythrocyte antioxidant level in Saanen goats.

In this study, by measuring malonyldialdehyde which is the sign of oxidative stress and some antioxidant parameters, the effect of aging, towards oxidants and antioxidant values in goat erythrocyte is shown.

Material and methods. During the present research, the effects of aging on erythrocyte oxidant-antioxidant values were investigated on 15 kids aged 3–4 month and 15 mother goats aged 4–5 years. Samples were collected into evacuated tubes (Vacutainer) with EDTA and without EDTA (for metabolic parameter determinations). All tubes for plasma collection were immediately placed on ice and within 2h of bleeding, 500 µL of each sample were centrifuged at 2500 g for 10 min at 4 ^{o}C the supernatant plasma was stored at -80 ^{o}C until analysis. Tubes for serum collection were allowed to clot at room temperature for 3h before centrifugation at 2000 g for 20 min, and sera was frozen at -20^{o}C until analysis. The hemoglobin content was determined using the cyanmethemoglobin method (Fairbanks and Klee, 1994).

The lipid peroxidation of serum was measured by the Tris –Boric Acid (TBA) (the method described by Yoshioka et al. (Yoshioka et al. 1979). MDA, formed from the breakdown of polyunsaturated fatty acids, was considered as an index for the peroxidation reaction. The absorbance of the action product of MDA with TBA was measured at 532 nm. Quantization was based upon a molar extinction coefficient of 1.56×105 M^{-1} cm^{-1}.

The activity of erythrocyte catalase (CAT) was measured according to the method of Aebi (Aebi 1984). The principle of the rate constant (s^{-1},k) of hydrogen peroxide (H_2O_2) decomposition by CAT. The rate constant was calculated from the following formula: k (2.3/8t) (a/b) log (A_1/A_2). In this formula, A_1 and A_2 are the absorbance values H_2O_2 at t_1 (0^{th} s) and t_2 (15^{th}s), a is the dilution factor, b is the hemoglobin content of erythrocytes. CAT activities were expressed as k/g of hemoglobin (k: rate constant of the first order reaction).

Erythrocyte glutathione peroxidase (GSH-Px) activity was determined by the coupled assay of Paglia and Valentine using t-butylhydroperoxide as substrate (Paglia and Valentine, 1967). The decrease in NADPH was recorded at 340 nm and them absorptivity of NADPH. 6.22×10^3 L/mol^{-1} cm^{-1} was used to calculate the enzyme activity. GSH-Px activity was expressed as units/g of hemoglobin (Oneunit, U: 1 µmol of NADPH transformed/min).

Erythrocyte GSH concentration was assayed by the method of Beutler et al. (Beutler et al. 1963)

The serum ceruloplasmin levels were determined by measuring p-phenylenediamine oxidase activity as described previously by Sunderman and Nomoto (Sunderman and Nomoto, 1970). Briefly, 5 ml phenylenediamine substrate (pH5.6) was added to the curve and test tubes. One microlitre sodium azide solution was then added into the curve tube only. This was followed by the addition of 0.1ml of sera to both the curve and test tubes. Samples were mixed and kept at 37.8 °C for 15 min. Finally, 1 ml of the sodium azide solution was added to the test tube only, and all samples were then incubated at room temperature for 15 min. The optical density was measured at 546 nm using a spectrophotometer (Shimadzu, UV-160).

Plasma vitamin C concentrations were determined spectrophotometrically by the colorimetric method (Kway, 1978). The levels of β-carotene in serum samples were determined according to the method of Suzuki and Katoh (Suzuki and Katoh, 1990). One ml serum was placed in a dark brown test tube. The serum was added 1.0 ml ethanol, then hexane and the tube was shaken mechanically for 10 min. All tubes were centrifuged at 800 g for 10 min. After centrifugation 3 ml of hexane was removed from end tubes and absorbance measured at 453 nm in a spectrophotometer (Shimadzu 1601).

The data was analysed using the Student's t-test (SPSS Version 12.0), and $P<0.05$ was considered as statistically significant.

Results and discussion. Averages of enzymatic and nonenzymatic parameters in mother and child Saanen goats are reported in Table 1. As serum MDA, erythrocyte GSH and GSH-Px levels in mother goats were found to be higher than kid goats ($P<0.05$), CAT levels were determined to be low ($P<0.05$). In plasma, the vitamin C and serum ceruloplasmin levels between mother and kid goats bore no difference ($P>0.05$), On the other hand, serum β-caroten concentration were determined to be higher in mother goats than kid goats ($P<0.05$).

Table 1. **Mean of enzymatic and non-enzymatic antioxidant parameters in Saanen goats**

	Mother Goats (X ± SD) (n: 15)	Kids Goats (X ± SD) (n:15)	P
MDA (µmol/L)	33.64 ± 3.61*	13.97 ± 1.12*	*
GSH-PX (IU/g-Hb)	410.09 ± 65.64*	220.17 ± 11.26*	*
CAT (k/g-Hb)	19.16 ± 2.76*	58.36 ± 5.61*	*
GSH (mmol/L)	4.42 ± 0.39*	2.46 ± 0.19*	*
Ceruloplasmin (mmol/L)	22.33 ± 1.16	22.54 ± 1.05	NS
β-carotene (µmol/L)	23.48 ± 1.05*	10.43 ± 0.42*	*
Vit C (mmol/L)	0.18 ± 0.027	0.20 ± 0.02	NS
X±SD = Mean ± Standart Error * – P<0.05; NS – Non-significant			

Recently, oxidative damage is thought to play a role in explaining the aging process. Oxidative damage is expressed to speed up the aging event and play an important role in pathogeneses of a series of illnesses that spring with aging (Geyikli et al. 2013).

With the oxidative damage, it is expressed that metabolism affects the aging process and if it is slowed down aging slows down, if it speeds up, oxygen consuming increases. As a result, an increase in free oxygen radicals occurs. Thus, radicals speed up aging by affecting the biomolecules (Balaban et al. 2005). In this study, the level of serum malonyldialdehyde (MDA) was determined to be significantly higher in mother goats than kids ($P<0.05$). This finding is in line with the reports of earlier investigators (Kasapoglu and Ozben, 2001; Aydilek and Simsek 2006; Suski et al. 2011). Increase of oxidative stress in old animals is expressed as derived from the increase of the mitochondrial free radical production with aging (Aydilek and Simsek, 2006; Suski et al. 2011).

The cause of higher oxidative stress during aging process is thought to be indicative of weakening of antioxidant defence systems. Antioxidative, which plays an important role in continuing life events in a healthy way in metabolism, aims to prevent occurrence of free radicals or hazardous effects on metabolism, thus names the molecules and enzymes taking place in the defence system developed by the organism. Recently, antioxidants also have been identified as intracellular, membranes and extracellular antioxidants. (Aydilek and Simsek, 2006).

As in the study by Kasapovic et al. (2010) erythrocyte GSH levels in mother goats were determined to be higher than in kid goats ($P<0.05$),the GSH levels in old people were determined to be higher than in young people and these results support our study. Antioxidant enzymes such as GSH-Px and CAT protect biological macromolecules from oxidative damages. GSH-Px turns the organic hydroperoxides and H_2O_2 into water and oxygen whereas catalase takes office in defence system by turning H_2O_2 into water and oxygen (Halliwell and Gutteridge, 1996). In previous studies of GSH-Px activity changes with related to aging, different results were obtained. Aydilek and Simsek, 2006 have reported that GSH-Px activity in old horses increases. On the other hand, according to Gaal et al (2006) the GSH-Px activity in old cows decreases. In this study, GSH-Px activity in mother goats were found to be higher than in kid goats ($P<0.05$). Oxidative stress in aging processes is thought to increase enzymatic activities of GSH and GSH-Px enzymes (Sinitsyna et al. 2006). Catalase enzyme levels of mother goats were found to be lower than in kid goats ($P<0.05$) and this finding is in line with the reports of earlier investigators (Saini et al. 2009; Kasapovic et al. 2010; Bagh at al 2011). High free radicals levels were reported inactivate this enzyme in aging process.

Besides intracellular antioxidant enzymes, measurements of the plasma and serum levels of extracellular antioxidants were carried out (Table 1). β-Carotene, in addition to peroxide radical, affects preventing the peroxidation process that is caused by O_2. The ability of β-Carotene terminating O_2, depends on the number of conjugated double bond that it contains 9 or more double bonds. B Carotene has 11 conjugated double bonds (Palozza and Krinsky, 1992). While same studies done by Aydilek et al (2006) on β-Carotene levels in old horses have been reported to be high, Sawada et al (1987) have reported no important difference in old and young rats plasma values. In the present study, β-Carotene levels of mother goats were found to be higher than kid goats and show increasing tendency of β-carotene. This can be evaluated as increasing utilization of vitamins indicating organism's adaptation to aging process (Hollander and Dadufalza, 1990).

Vitamin C has some missions such as catching hydroxyl and singlet oxygen, cleaning superoxide anion (O_2^-) and peroxyl radicals that dissolve in water, reducing carcinogenic nitrosamines to inactive products, protecting plasma lipids towards the peroxidations that active notrofills cause (Halliwell and Gutteridge, 1990). Arivazhagan et al. (1999) have defined vitamin C levels to be low in old rats. In our study, it was determined that, vitamin C levels in mother goats were lower than kid goats and statistically not meaningful, at the same time vitamin C concentrations did not change in relation to aging ($P>0.05$).

The mission of ceruloplasmin which is a α_2-glikoprotein is not only carrying copper. Among the other features its oxidation effects towards ferrous ions must be pointed out. It oxidises Fe^{+3} to Fe^{+2}. and converts oxygen into water. Ferroxidase activity provides inhibition of lipid peroxidation connected to ferrous ion and formation of HO from H_2O_2. After Fe^{+2} turns into Fe^{+3}, it conjoins tightly transferring. Even if there is no transferring, ceruloplasmin serves as a powerful antioxidant (Pacht and Davis, 1988). Gümüşlü et al. (2000) have determined that ceruloplasmin levels in old rats are significantly higher and than in young rats. In our study, no difference in their ceroplasmin levels was determined between mother and kid goats ($P>0.05$).

Conclusion. Consequently, while the level of serum MDA was higher significantly in mother goats than kids, serum ceruloplasmin and plasma vitamin C levels were protected. Towards the increased oxidative stress connected to aging; while GSH and β-carotene which are one of the non-enzymatic antioxidants and GSH-Px concentrations which are one of the enzymatic antioxidants were determined to be higher in mother goats than kid goats, CAT enzyme activity was determined to be lower in mother goats than kid goats. In short, enzymatic and non enzymatic antioxidant defence systems in Saanen goats can be said to be affected from aging process.

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