Asian-Aust. J. Anim. Sci.
Vol. 19, No. 9 : 1262 - 1265
September 2006

www.ajas.info

Status of Lipid Peroxidation in Normal Cycling and α-Tocopherol Supplemented Anestrus Buffalo Heifers (*Bubalus bubalis*)

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ABSTRACT : The present study was undertaken to analyze the status of erythrocytic malonyl dialdehyde (MDA) as an index of lipid peroxidation in normal cycling and α-tocopherol supplemented anestrus buffalo heifers. Before supplementation, the blood samples were collected at weekly interval in anestrus and normal cycling buffalo heifers and data from four samples was pooled to establish pre-supplementation base line. Thereafter, the blood samples from anestrus and α-tocopherol supplemented anestrus buffalo heifers were taken at fortnightly interval for 12 weeks during supplementation and for four weeks during the post-supplementation period. The pre-supplementation endogenous erythrocytic MDA, 0.3% H_2O_2 induced-erythrocytic MDA and 1.5% H_2O_2 induced-erythrocytic MDA levels (nmol/g Hb) increased significantly ($p<0.05$) in anestrus heifers (372.3±5.0, 564.4±5.7, 862.4±8.5) when compared to normal cycling animals (289.6±3.0, 508.2±6.0, 777.7±4.9). Oral supplementation of 3,000 mg of α-tocopherol per week per animal in anestrus heifers resulted in a progressive and significant ($p<0.01$) decline in erythrocytic MDA levels. These observations implied that supplementation of α-tocopherol to anestrus buffalo heifers ameliorated their antioxidant status by alleviating the effects of oxidative stress. (**Key Words :** Lipid Peroxidation, Malonyl Dialdehyde, α-Tocopherol, Anestrus, Buffalo Heifers)

INTRODUCTION

Lipid peroxidation is a free radical chain reaction, which causes oxidative damage to membrane lipids in biological systems. It impairs the biological functions of membrane, inactivates membrane bound enzymes and receptors and may change nonspecific calcium ion permeability (Orrenius et al., 1989; Aruoma, 1998). The basic pre-requisite for the occurrence of lipid peroxidation is inadequate free radical scavengers.

Brzezinska-Slebodzinska et al. (1994) studied antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium and observed that thiobarbituric acid reactive substances (TBARS) were correlated negatively with plasma total antioxidants and α-tocopherol. The deficiency of vitamin E and selenium stimulated lipid peroxidation and peroxidative challenge in muscles of calves that was involved in the pathogenesis of nutritional degenerative myopathy (Walsh et al., 1993a, b). Dietary vitamin E supplementation delayed metmyoglobin increase and highly suppressed lipid peroxidation in beef as compared to control (Mitsumoto et al., 1995; Roeder et al., 1996). The oral supplementation of α-tocopherol in anestrus heifers decreased erythrocytic superoxide dismutase and glucose-6-phosphate dehydrogenase activities significantly ($p<0.01$) but with non-significant increase in erythrocytic glutathione peroxidase activity (Kahlon and Singh, 2003). Kahlon et al. (2003) showed a significant linear relationship between weeks of supplementation and plasma T_3 levels in anestrus animals. Supplementation of α-tocopherol to anestrus buffalo heifers improved the antioxidant status by mitigating the harmful effects of free radical induced oxidative stress (Kahlon and Singh, 2004).

Perusal of available literature revealed that information regarding role of vitamin E on lipid peroxidation in anoestrus buffaloes is inadequate. Therefore, the present trial was undertaken to analyze the lipid peroxidation in normal cycling and α-tocopherol supplemented anestrus buffalo heifers.

MATERIALS AND METHODS

Experimental animals

The investigation was conducted on 13 clinically

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Received December 16, 2004; Accepted September 28, 2005

Table 1. Erythrocytic malonyl dialdehyde (MDA) levels in normal cycling and α-tocopherol supplemented anoestrus buffalo heifers (mean±SE)

Groups	Weeks of supplementation							Weeks of post supplementation	
	0	2	4	6	8	10	12	2	4
Endogenous erythrocytic MDA (nmol/g Hb)									
Anoestrus	372.3^{a}±5.00	328.7^{a**}±6.0	308.7^{a**}±7.2	290.8^{a**}±8.2	246.4^{a**}±11.0	217.5^{a**}±4.9	197.6^{a**}±8.8	192.3^{a**}±5.5	195.00^{a**}±6.2
Anoestrus control	367.3^{a}±7.0	387.7^{b}±8.5	377.7^{b}±5.3	370.5^{b}±11.4	376.8^{b}±13.4	370.3^{b}±17.9	370.5^{b}±13.6	372.5^{b}±15.6	377.3^{b}±18.8
Normal cycling	289.6^{b}±3.0	285.7^{c}±7.1	277.3^{c}±8.4	276.5^{a}±10.2	266.0^{a}±7.2	267.6^{c}±11.6	266.3^{c}±9.6	260.6^{c}±11.8	259.1^{c}±19.0
H_2O_2 (0.3%) induced erythrocytic MDA (nmol/g Hb)									
Anoesrtus	564.4^{a}±5.7	522.5^{a}±7.5	476.4^{a**}±11.5	463.0^{a**}±9.2	419.6^{a**}±10.8	414.9^{a**}±11.6	395.1^{a**}±14.1	390.3^{a**}±15.0	390.1^{a**}±16.6
Anoestrus control	572.18^{a}±3.16	577.03^{b}±9.32	575.20^{b}±6.11	568.05^{b}±2.04	564.49^{b}±6.56	563.14^{b}±13.04	560.41^{b}±9.29	553.07^{b}±10.62	552.62^{b}±9.78
Normal cycling	508.2^{b}±6.0	501.2^{a}±4.1	497.1^{a}±8.9	483.3^{a}±7.2	478.2^{c}±8.1	479.4^{c}±8.1	483.3^{c}±9.4	454.3^{c}±10.5	479.1^{c}±8.2
H_2O_2 (1.5%) induced erythrocytic MDA (nmol/g Hb)									
Anoesrtus	862.4^{a}±8.5	836.2^{a}±6.5	811.4^{a**}±6.5	779.5^{a**}±8.3	718.2^{a**}±7.3	703.8^{a**}±6.2	677.0^{a**}±7.3	675.9^{a**}±7.4	679.3^{a**}±7.3
Anoestrus control	867.7^{a}±12.7	852.4^{a}±6.2	849.8^{b}±4.4	860.7^{b}±6.4	855.8^{b}±9.2	849.3^{b}±9.3	845.2^{b}±7.2	847.7^{b}±4.7	845.9^{b}±2.9
Normal cycling	777.7^{b}±4.9	774.8^{c}±7.8	774.5^{c}±9.5	776.2^{a}±11.9	768.3^{d}±13.0	752.7^{c}±13.9	749.5^{d}±9.0	750.8^{d}±8.3	749.8^{d}±11.2

The values having same superscripts within a parameter column don't differ significantly ($p<0.05$) from each other.
The values having asterisk (** $p<0.001$; * $p<0.05$) within a row differ significantly from pre supplementation value.

healthy Murrah buffalo heifers between 2 to 4 years old and having more than 250 kg body weight. These animals were maintained as per standard feeding and managemental conditions practiced at the dairy farm of Punjab Agricultural University, Ludhiana, India (Latitude 30° 45′; Longitude 75° 48′). Buffalo heifers were selected on the basis of their reproductive history and status of reproductive organs as assessed by rectal examination before commencement of study.

Selection of antioxidant

α-Tocopherol was selected for supplementation because it is a non-toxic antioxidant and its toxicity has not been reported so far.

Grouping of animals

The buffalo heifers after selection were divided into 2 groups as follows:

Anestrus group : Eight buffalo heifers with inactive and smooth ovaries and showing sexual quiescence for at least three preceding reproductive cycles were selected in this group. Five animals were supplemented orally with 3,000 mg α-tocopherol (as acetate) per animal per week for 12 weeks and remaining 3 animals were kept as control. The amount of α-tocopherol supplementation was based on requirements of vitamin E as described by Putnam and Comben (1987).

Normal cycling group : Five buffalo heifers, showing normal estrus cyclicity during two preceding estrus cycles, were selected in this group.

Sampling schedule

The blood samples of anestrus and normal cycling buffalo heifers were collected at weekly interval and data of four samples was pooled to establish pre-supplementation base line.

The blood samples were collected at fortnightly interval for 12 weeks in normal cycling and α-tocopherol supplemented anestrus heifers.

The blood samples were collected in both groups at fortnightly interval for 4 weeks during post-supplementation period.

Sampling

Blood samples were collected aseptically from jugular vein in heparinized glass stopper vials and processed for preparation of hemolysate. After preparation of hemolysates, lipid peroxidation was estimated within four hours.

Biological procedures

Lipid peroxidation was assayed by method of Placer et al. (1966). The method is based on the principle that the reaction of malonyl dialdehyde (MDA), an end product of lipid peroxidation, with thiobarbituric acid (TBA) yielded a pink coloured trimethine complex exhibiting an absorption maximum at 548 nm.

Statistical analysis

Data was subjected to one way analysis of variance (ANOVA) on computer using GraphPad InStat Programme developed by Peter Russell, Royal Veterinary College London 9508375. Regression analyses were carried out using Microsoft Excel programme.

RESULTS AND DISCUSSION

The mean endogenous erythrocytic MDA levels and

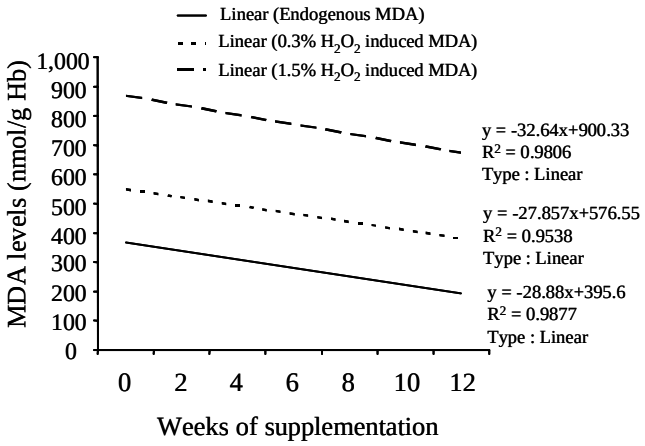

Figure 1. Regression analysis of erythrocytic MDA levels in α-tocopherol supplemented anestrus buffalo heifers.

H_2O_2 induced- erythrocytic MDA levels in normal cycling and α-tocopherol supplemented anoestrus buffalo heifers are summerized in Table 1.

The endogenous as well as H_2O_2 activated erythrocytic MDA levels in normal cycling heifers were significantly ($p<0.05$) lower as compared to those in anoestrus buffalo heifers (Table 1). Supplementation with α-tocopherol in anoestrus buffalo heifers decreased erythrocytic MDA levels, which was progressive and significant ($p<0.01$). After 12th week of α-tocopherol supplementation, the maximum decline in endogenous and 0.3% and 1.5% H_2O_2 induced-erythrocytic MDA levels was 46.91% (197.6 vs. 372.3), 30.00% (395.1 vs. 564.4), 21.49% (677.0 vs. 862.4) in anoestrus heifers as compared with respective pre-supplementation levels. A significant ($p<0.05$) linear regression between weeks of α-tocopherol supplementation and erythrocytic MDA levels was observed in anoestrus buffalo heifers (Figure 1). There was a significant ($p<0.05$) inverse correlation between plasma vitamin E levels and endogenous erythrocytic MDA levels (-0.8192), 0.3% H_2O_2 induced-erythrocytic MDA levels (-0.9620) and 1.5% H_2O_2 induced-erythrocytic MDA levels (-0.8236) in α-tocopherol supplemented anoestrus heifers.

When incubated with solutions of H_2O_2, erythrocytes of stress-susceptible pigs produced more products of lipid peroxidation than erythrocytes from stress-resistant pigs (Duthie et al., 1989). Supplementation of vitamin E for 6 weeks in prepartum dairy cows decreased 27-28% of endogenous lipid peroxidation in red blood cells (Brzezinska-Slebodzinska et al., 1994). Walsh et al. (1993a, b) also reported lower muscle concentrations of indices of lipid peroxidation in vitamin E supplemented calves than deficient ones.

Unsaturated fatty acids, particularly the polyunsaturated fatty acids (PUFA) are the component of tissue membranes including erythrocytic membrane (Thompson, 1980). The

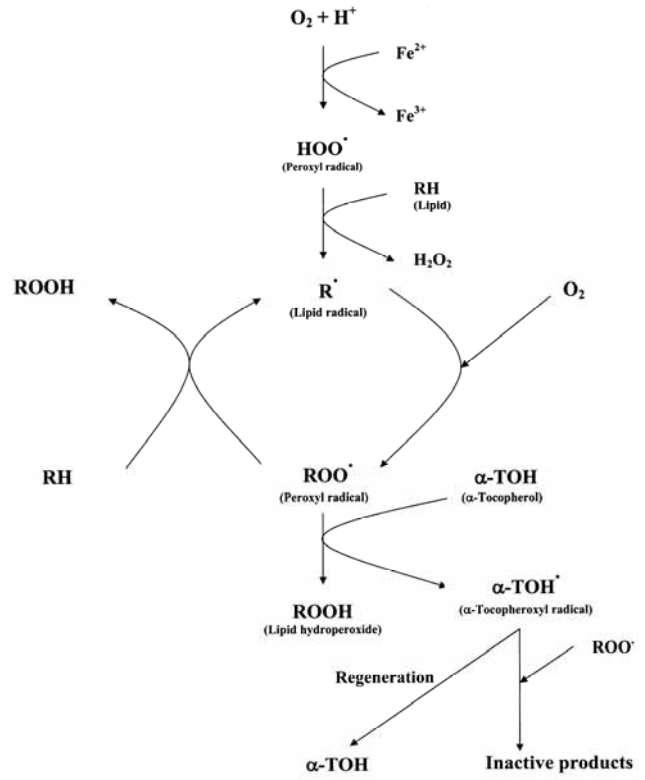

Figure 2. Chain breaking antioxidant action of α-tocopherol on lipid peroxidation (Kahlon et al., 2001).

unsaturated double bonds of membrane PUFA are inherently unstable and readily attacked by peroxides and other forms of reactive oxygen species (ROS) (Putnam and Comben, 1987). This process tends to produce a chain reaction called lipid peroxidation (Diplock, 1983) and more free radicals and hydroperoxides are formed (Figure 2). The α-tocopherol acts as a scavenger of free radicals and prevents this runway chain reaction (Burton and Traber, 1990; Rock et al., 1996). α-Tocopherol short-circuits this destructive propagative cycle and can intercept the peroxyl radicals (ROO) more rapidly than can PUFA. α-Tocopherol donates its phenolic hydrogen atom to the radical and yields a hydroperoxide and α-tocopheroxyl radical, which is sufficiently stable to be unable to continue the chain (Liebler and Burr, 1995) and, instead, is removed from the cycle by reaction with another peroxyl radical to form inactive non-radical product or regenerated back to α-tocopherol (Chan, 1993).

Increased lipid peroxidation of erythrocytes in anoestrus buffalo heifers indicated the presence of oxidative stress in these animals. This augmented lipid peroxidation anoestrus heifers could also be due to stimulated the permeability and fluidity of erythrocytic membrane, which contributes to their enhanced osmotic fragility (Kahlon et al., 2002) and hence oxidative stress. Therefore, consumption of NADPH by ROS reactions (Golden and Ramdath, 1987) and

susceptibility of steroidogenic enzymes to lipid peroxidation (Staats, 1988; Takayanagi et al., 1986) can limit synthesis of steroid hormones under the stress of free radicals. Therefore, metabolic changes mediated by ROS and peroxidative inactivation of steroidogenic enzymes may contribute to anoestrus in buffalo heifers. The decline in erythrocytic lipid peroxidation during supplementation in the present study was due to chain breaking antioxidant action of supplemented α-tocopherol, which augmented the antioxidant status by lessening the harmful effects of free radical induced oxidative stress.

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