EVALUATION OF OXIDATIVE STATUS IN TRANSITION DAIRY COWS

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Abstract

Dairy cows are considered to be prone to oxidative stress during the period from late pregnancy to onset of lactation, i.e., during the transition period. In this study, status of oxidative stress of 16 crossbred dairy cows was evaluated, from 8 weeks before calving till 8 weeks after calving, at fortnightly intervals using two indices in the serum: Malondialdehyde (MDA, nmoles/ml) and Total Antioxidant Status (TAS presented as TEAC). The mean values of MDA and TAS did not reveal significant difference between different time periods. But, the mean MDA values were higher during transition period (9.07±1.72) when compared to outside transition period (5.59±0.55) with a p value of 0.06. TAS remained fairly constant during the period. Though the indicators of oxidative stress did not show significant variation, the oxidative status could not be considered optimum as eleven out of sixteen animals studied suffered from ketosis, hypocalcaemia or mastitis during the study period.

Keywords: Transition period, Oxidative stress, MDA, TAS, Dairy cows

I. INTRODUCTION

Transition period, the period between three weeks before parturition until 3 weeks after it (1), is considered as the most critical period during the productive life of all dairy cows and is characterized by endocrine, immune and metabolic changes (2). This, along with a shift to the lactation diet and reduced feed intake accompanying parturition often results in energy imbalance which makes the animal prone to many diseases like ketosis, mastitis, metritis etc (3). The intense metabolic processes associated with pregnancy and lactation in dairy cows results in generation of free radicals. Though free radicals are normally generated in the body as a result of various metabolic processes, these are counteracted by the antioxidants, which is body’s oxidant defense mechanism. The rate of free radical production is increased during bacterial infections and certain pathological conditions. The animal is also exposed to various exogenous oxidants like radiations, toxins from different chemicals like disinfectants etc which also increases the free radical production in the body (4). In transition dairy cows the increased rate of metabolism results in production of free radicals at a level greater than that can be counteracted by the body’s defense mechanism and this results in oxidative stress (5). The free radicals have direct effect on biomolecules like nucleic acids, membrane lipids and proteins which may cause altered metabolism or dysfunctional pathways. Thus the oxidative stress during transition period is an underlying cause of various health problems, especially in high yielding dairy cows.

II. MATERIALS AND METHODS

A. Animals and feeding

The study was conducted in 16 clinically healthy pregnant crossbred dairy cows in their second to fourth parity maintained in University Livestock Farm, College of Veterinary and Animal
Sciences, Mannuthy and the Livestock Research Station, Thumburmuzhy of Kerala Veterinary and Animal Sciences University during the period from November, 2016 to April, 2017. Animals were fed with concentrate containing 18 % crude protein and supplemented with a mineral mixture containing Ca, Mg, Cu, Co, Fe, Zn, Mn and I. Green fodder was provided ad lib. for lactating animals and not less than 25 kg for pregnant animals. The average milk yield of the animals under study was around 12 kg/day. The climatic conditions were relatively stable throughout the study period.

Blood was collected aseptically from the jugular vein at time points 8, 6, 4 and 2 weeks before predicted date of calving (-8, -6, -4, -2), 24 h within parturition (0) and 2, 4, 6 and 8 weeks post calving (+2, +4, +6 and +8), using a sterile needle. Approximately 10 ml of blood was collected from each animal and transferred to a vial without any anticoagulant. The blood was allowed to clot and the serum was separated by centrifugation at 3000 rpm for 15 minutes and was stored at -40°C until further investigations were made.

B. Materials

The measurements of MDA and TAS were made using UV/VIS spectrophotometer (Perkin–Elmer) using the standards 1, 1, 3, 3-Tetramethoxypropane (TMP) from HiMedia Laboratories Pvt. Ltd. for MDA and Trolox (6-hydroxy-2, 5, 7, 8-tetmethylchroman-2-carboxylic acid; Sigma Aldrich Co., USA) for Total Antioxidant Status (TAS).

C. Estimation of plasma malondialdehyde (MDA)

Concentration of MDA was determined by a spectrophotometric assay as described by Yagi (6). The method was based on the reaction of MDA with thiobarbituric acid (TBA) forming a red coloured adduct. Briefly, 200 μl of serum was added into the reaction mixture containing 4 ml N/12 H₂SO₄ and 0.3 ml of 10% phosphotungstic acid. The reaction mixture was incubated at room temperature for 5 min, centrifuged and supernatant discarded. The pellet was resuspended in 4 ml distilled water and 1ml of TBA reagent was added. The mixture was kept in water bath maintained at a temperature of 95°C for 60 min. After cooling in tap water, 5 ml of n-butanol was added and centrifuged at 3000 rpm for 15 min after vigorous shaking. The absorbance of the butanol layer was measured at 532 nm by keeping n-butanol as blank. The results were estimated from a standard graph using differed concentrations of TMP. The concentration of MDA was calculated using the formula:

\[
\text{Level of MDA (nmol/ml of serum)} = \frac{a \times 0.5}{A \times 0.2}
\]

Where \(a\): Absorbance of the sample, \(A\): Absorbance of the standard, 0.5: Concentration of the standard solution and 0.2 ml: Volume of sample taken

D. Estimation of total antioxidant status (TAS)

TAS in the serum was measured using a modified decolorizing assay developed by Re et al.(7). 7 mM 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2.45 mM potassium persulfate in distilled water were mixed in the ratio of 1:1. The reaction mixture was kept overnight in dark generating monocation radical of ABTS (ABTS+), which is a blue/green chromophore with absorption maxima at 734 nm. The ABTS+ solution was diluted with phosphate buffered saline, pH 7.4, to an absorbance of 0.70 (±0.02) at 734 nm by keeping PBS as blank. One ml of diluted ABTS+ was mixed with 10 μl of test sample and the reduction in absorbance was measured exactly after 1 min. The method was repeated with distilled water as control and the % inhibition was calculated based on the formula

\[
\text{% inhibition} = \times 100
\]

\[
\text{Absorbance of control} - \text{Absorbance of sample}
\]

\[
\frac{\text{Absorbance of control}}{}
\]
The method was repeated with different concentrations of Trolox as standard. Trolox (2.5 mM) was prepared in phosphate buffered saline, pH 7.4 for use as stock standard. Different concentrations of the standard were prepared from the stock by diluting with PBS. The reduction in absorbance was measured and the % inhibition was calculated. A graph was plotted as a function of concentration of Trolox and % inhibition for the standard reference data (Fig.1). The concentration of the antioxidants in the sample was obtained by comparing the values with the standard curve prepared and was expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

III. RESULTS AND DISCUSSION

There are different methods for assessing the oxidant status, electron spin resonance being considered the gold standard. Determination of plasma reactive oxygen species have been used successfully by several authors for determining the redox status of animals. In addition, products of free radical attack on macromolecules, such as advanced oxidation protein products and malondialdehyde (lipid peroxidation product) have been employed as biomarkers of oxidative stress. Organisms are armed with several antioxidant substances to deal with oxidant attacks. Assessment of total antioxidant activity gives a better reflection of antioxidant status than estimation of individual antioxidants as many of the antioxidants act synergistically and are often reciprocally compensated.

MDA and TAS are reported to provide an accurate reflection of the oxidant status of the animal and are good indicators of oxidative stress in dairy cows during late pregnancy and early lactation (8).

The the indices of oxidative status of the animal are given in Tables 1 and 2. Statistical analysis was done using the software SPSS Version 24.0. Comparison of the status during the transition and outside was done by using paired t-test.

| Table 1: The concentration of MDA (nmoles/ml) at the observed time periods |
|--------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Group | -8 weeks | -6 weeks | -4 weeks | -2 weeks | 0 weeks | +2 weeks | +4 weeks | +6 weeks | +8 weeks |
| MDA (µM) | 6.630±1.15 | 5.882±1.00 | 5.133±1.00 | 7.298±1.84 | 6.751±1.54 | 9.615±2.54 | 5.375±0.91 | 5.100±0.94 | 4.993±0.72 |

| Table 2: TAS in terms of TEAC at the observed time periods |
|--------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Group | -8 Weeks | -6 Weeks | -4 Weeks | -2 Weeks | 0 Weeks | +2 Weeks | +4 Weeks | +6 Weeks | +8 Weeks |
| TAS (TEAC) | 0.894±0.061 | 1.020±0.056 | 0.891±0.061 | 0.924±0.057 | 0.951±0.071 | 0.953±0.052 | 0.984±0.058 | 1.005±0.040 | 0.933±0.064 |

Concentration of MDA did not show any significant difference between the observed time periods. But the values observed during transition period (-2, 0 and +2) showed a higher mean value (9.07±1.72) when compared to that observed outside the transition period. The highest value was observed at 2 weeks after calving (9.615±2.54). No significant difference was observed for TAS also between different observed time points.

Though the difference between the observed time periods was not seen significant it can be observed that the concentration of MDA showed an increase during the transition period (-2, 0 and +2 weeks) which might be the result of increased oxidants generated by hypermetabolic (catabolic) response to the changes in homeostasis evoked by parturition and lactation. This could result in imbalance between oxidants and antioxidants thus generating an oxidative stress (9). A decreasing trend in the concentration of MDA could be noted outside the transition period, post calving. It could be assumed that the animal’s body gets adapted to the metabolic alterations gradually maintaining a
homeorhetic balance. The results are in agreement with Castillo et al. (10) who reported increased lipid peroxidation around parturition but with high variation between individual cows. Sharma et al. (11) reported significant increase in the concentration of MDA in early lactating cows compared to the late pregnant ones when a group of pregnant animals were compared to a group of early lactating animals. Castillo et al. (8) has reported that there was no significant difference in plasma MDA concentration between animals in different stages of lactation.

The total antioxidant activity remained relatively constant throughout the study period. According to Castillo et al. (8), it is not necessarily a desirable condition to have an increase in TAS value due to adaptive oxidative stress response and also is not undesirable to have decreased value of TAS if the production of reactive oxygen species is less. It appeared that the total antioxidant activity was not optimum during the study period as eleven out of the sixteen animals studied suffered from ketosis, endometritis, mastitis or hypocalcaemia during the period though the milk yield was not affected strikingly. Sordillo et al. (12) reported that the changes associated with parturition results in a loss in overall antioxidant potential and this could compromise the animal’s immunological defenses resulting in increased incidence of diseases during the transition period. Further studies are required to ascertain if supplementation of antioxidants would improve the health status of the animal and protect it from production diseases during transition.

Though all the animals under study were reared under the similar feeding and management practices the variation in the studied parameters was high indicated by a high coefficient of variation. The high inter-individual variation within the group could be due to the differences in the adaptive capacity of individual animals to optimize the oxygen consumption to neutralize the free radicals generated (13).
IV. CONCLUSION

The level of MDA and TAS assessment provide good complementary tools in assessing the oxidative status of the animal. Dairy cows are seemed to be under more oxidative stress during 2 weeks before and after calving which are of short term and the animal gradually gets adapted to these stressful conditions. The nutritive status of the animal must also be taken into consideration while measuring the total antioxidant capacity.

BIBLIOGRAPHY