The influence of solar radiation on the antioxidant systems in blood of dairy cows and the processing of the data using wavelets transform

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INTRODUCTION

This paper presents some of the results belonging to a larger study which wants to find out if the milking cows are submitted to the heat stress induced by the solar radiation.

On this purpose there were previously determined the main physiological indexes, haematological indexes, the level of the thyroid hormones and in the end, the level of the antioxidant systems in blood.

The preliminary studies demonstrated that the month May represents the month for thermal comfort in cattle, which was considered in the study as reference month and August was the month when the cows were submitted to the most increased thermal stress.

THE PURPOSE OF THE PAPER

Is to observe if in dairy cattle, exposed to the solar radiation, could be noticed a certain reaction of the organism related to the oxidative stress, manifested by the increasing of the level of the antioxidant enzymes such as: superoxide dismutase (SOD), catalase and peroxidase in August comparing to May and the processing of the data using wavelets transforms.

GENERAL ASPECTS

First of all, in order to have a better view, we present some considerations regarding the antioxidant systems in blood and ORS (Oxygen Reactive Species). The main ORS are: singlet oxygen, superoxyde radical anion, hydrogen peroxide and their radicals.

Normally, the organism is protected by a large series of enzymatic antioxidants systems, which interact together. These are mainly represented by superoxide dismutase, catalase and peroxidases. These antioxidants systems have the role to control the forming and accumulation in the organism of the oxygen reactive species (ORS).

ORS may be formed in organism, both endogen and exogenous under the action of some physical or chemical agents. *[Dejica, 2000].*

GENERAL ASPECTS

The solar radiation acts, also directly and also indirectly by increasing the values of environmental temperatures. The most common index of heat stress (Temperature – Humidity Index - THI) is calculated using temperature and relative humidity (Yousef, 1967).

THI= (1.8xT+32) – [(0.55-0.0055xRH) x (1.8xT-26)] where T=Temperature and RH=Relative Humidity

Dairy cows begin to suffer whenever the THI exceeds 72 (Jones, 1999: West, 1995, 2000). Under these circumstances we recorded the forming, respective the accumulation in excess of the ORS which determines, so called "oxidative stress".

The oxidative stress is produced by the increasing of the free radicals or ORS production and / or by the reduction of the antioxidant defence. (Travisan *et al. 2001, quot. by* Bernabucci *et al.* 2002).

When the forming of the ORS rate increases, so that they can not be further neutralised by the action of the antioxidants systems, the oxidative stress is produced (Sies, 1991, qout. by Bernabucci *et al.*, 2002).

MATERIAL AND METHOD

The study was realised on a group consisting in 16 Romanian Simmental milking cows, clinically health, kept on pasture during the day and in stable during the night.

On the purpose to establish the level of the antioxidant enzymes, there were taken from the cows blood samples on heparin, during the days of May and August.

The blood samples were taken at 20.00 o'clock, each time after the cows were submitted to the solar radiation.

We took in observation the same group of cows, comparing the data obtained in May, considered as control data, with those obtained in August, considered as experimental data.

MATERIAL AND METHOD

During the days of the experiment there were also recorded the meteorological data, such as: air temperature, relative humidity and there were calculated the maximum THI index values. The meteorological data registration was made in Meteorological Station belonging to USAMV Cluj-Napoca.

The determinations of the antioxidant enzyme's level were made in the Biochemistry Laboratory belonging to the Faculty of Veterinary Medicine Cluj-Napoca.

The determination of SOD was made using NBT method, based on pyrogallic oxidation in the presence of nitro blue tetrazolium chloride (NBT) resulting a stained product which can be photometric dosed at 540 nm (Ciurdaru V, 1999).

The catalase activity was made using phothometric metod with potasium bichromate (after Sinha A., 1972, adapted by Sanda Andrei 2004) which is based on the reaction of the hydrogen peroxide with potasium bichromate.

The peroxidase dosing was made using the guayacol method, which is based on the guayacol oxydation as hydrogen donor, resulting a coloured product which can be photometric dosed at 470 nm (Ciurdaru V, 1999).

MATERIAL AND METHOD

The obtained mean values of the SOD, of the catalase level and of the peroxidase activity were processed using wavelet transforms.

A wavelet transform (WT) decomposes a signal (i.e. a sequence of numerical measurements) into several groups (vectors) contain information about characteristics of the sequence at different scales.

Coefficients at coarse scales capture gross and global features while coefficients at fine scales contain local details.

Further it was explained the practical significance of wavelets coefficients considering the In-Place Fast Haar Wavelet Transform applied on real data.

The resulted data were also processed using ANOVA model implemented in R-Statistics.

RESULTS AND DISCUSSIONS

In May, the calculated values for maximum THI were lower than 72, all the time of the day, varying between 43.4 and 67.5, so they are within the limit for the thermal comfort in cows.

On the contrary, the maximum THI index obtained in August was 82-83, being increased with 10.27% over the limit of 72, which represents the limit over which the thermal discomfort appears in milking cows.

The experiments, previously made by us, presented an increasing of the main physiological indexes (such as respiratory rate, heart rate, internal and cutaneous temperature, variation of the blood indexes and thyroid hormones) determined us to study the reaction of the cows' organism at the cellular level, regarding the thermal stress.

RESULTS AND DISCUSSIONS

Values of the antioxidant enzymes level (mean ±SD) in August compared to May

	SOD Activity (U / ml)		Catalase Activity (Ncat / ml)		Glutation Peroxidase Activity (Px / ml)	
	May	**August**	**May**	**August**	**May**	**August**
Mean (X±SD)	30.57 ± 7.28	59.73 ± 20.70	1917.53 ± 1411.84	3434.18 ± 1930.55	70.58 ± 15.62	78.88 ± 7.95

RESULTS AND DISCUSSIONS

The values of the resulted wavelets coefficients

SOD Activity	**Catalase Activity**	Glutation Peroxidase
-14.58	**-758.32**	**- 4.61**

The p values of antioxidant enzymes

SOD Activity	**Catalase Activity**	Glutation Peroxidase
p=0.001<0.05	**p=0.017<0.05**	p=0.044<0.05

RESULTS AND DISCUSSIONS

In each case the transform generates $32=2^5$ coefficients, out of which it is to be taken into consideration the coefficient no.16, which present the variation of the values from May to August.

These coefficients show a general increase of the antioxidant enzyme levels, each value must be multiply with (-2) in order to present the variation of each type of antioxidant enzyme.

So, the average increase of the enzymatic values in August compared to May is 29.16 for SOD (an increase of 95%in August compared to the May average), 1516.64 for catalase (an increase of 79% in August compared to the May average) and 9.24 for glutation peroxidase (an increase of 13% in August compared to the May average).

RESULTS AND DISCUSSIONS

In the animal organisms, the forming of the ORS is **endogenous** (at the mithocondrial, peroxisomes and lysosomes levels) and also at the cellular and microsomal level (during the prostaglandine biosynthesis). (Jezek, 2005; Jackson, 2009, Turrens, 1997, 2003)

ORS are represented by the singlet oxygen, superoxyde radical anion, hydrogen peroxide and their radicals.

All ORS are **strong oxidants**, which determine the irreversible oxidative transformations of the cellular biomolecules. (Kierkman, 2007) These transformations were named **peroxidation** because they are initiated in the presence of the molecular oxygen and they are produced intermediated by peroxide derivates. In the organism these ORS have an action over the lipids, determining the so-called lipoperoxidation and they also have an action over the proteins, producing protein oxidation and over the nucleic acids. (Murphy, 2011).

RESULTS AND DISCUSSIONS

At the cellular level the forming and accumulation reactions of the ORS are **controlled by antioxidant enzymatic systems** which include **superoxide dismutase (SOD),** present as Mn-SOD in mithocondria; Cu and Zn-SOD in cytoplasm and extracellular by EC-SOD. (Iliukha, 2001). The **catalase and peroxidase** exist under glutation-peroxidase (GPx), lactoperoxidase (LPx), mieloperoxidase (MPx), cytochrom-peroxidase (cCPx) and thioredoxin peroxidase (TPx) (Nodberg, 2001).

These enzymes have different location in the cellular compartments. So that, the catalase is located in mithocondria and peroxisomes, where also act the glutation-peroxidase. In the cytoplasm the glutation-peroxidase is coupled with SOD.

In this way the protection of the sub-cellular structures is provided and in the same time the formation of the hydroxyl radicals is avoided. (Noori, 2012).

RESULTS AND DISCUSSIONS

The formation and the accumulation of the ORS determine the so-called „oxidative stress" which represents the totality of the oxidative degradation produced by the oxygen free radicals. (Olinescu, 1994).

The oxidative stress is produced by the increasing rate of free radicals production or ORS and/or by the decreasing of the antioxidant defence determined by metabolic disturbances. (Travisan 2001, quot. by Bernabucci, 2002).

So, when the ORS speed formation increase they can not be neutralized any longer by the antioxidant enzymes action and the oxidative stress is produced. (Sies, 1991, quot. by Bernabucci, 2002).

RESULTS AND DISCUSSIONS

The increasing of the **antioxidant enzymes level** proofs that, during the hot summer day, when the values of the **THI index are higher then 72**, which is the limit value for the thermal comfort **the cows were submitted to the heat stress.**

We can also surely say that between the increasing of the blood level of the antioxidants enzymes and the THI values exist a **direct correlated relation**, so that means that when the values of THI exceed 72, the production of the antioxidant enzymes at the blood level starts to increase.

The increasing of the antioxidants enzymes is due to the fact that in the animals organism the **ORS are accumulated in the organism**, by increasing the level of the antioxidant enzymes is trying to “neutralise” them. This can be explained by the **hyperventilation which determines hyperoxia at the cell level and which is produced in the conditions of heat stress in cows.**

Under the hyperoxia conditions is increased the level of lipids and protein oxidation, which determines the producing of ORS and as a consequence the **level of the antioxidant enzymes increases**.

CONCLUSIONS

1. During the hot summer days, when the values of THI exceed 72, the oxidative stress is produced in dairy cows, which is mainly manifested till the cellular level, by the increasing of the antioxidant enzymes activity.

2. The oxidative stress in milking cows is mainly manifested by the increasing of the SOD with 95% in August comparing to May, followed by the increasing of the catalase level with 79% and of the general peroxidase with 13%.

3. There was recorded a direct co-relation between the increasing of the antioxidant enzymes level and the increasing of the THI.

4. The increasing of the enzymes levels, which forms the antioxidant system, at the blood level, seems to represent in dairy cows an adaptative answer to heat stress.

5. The wavelets transform can be easily adapted to biological data, due to the fact that the coefficients are easy to be calculated, and the interpretation of the data may easier and faster be made compared to the classical statistical methods.

THANK YOU VERY MUCH
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