Examination of the redox status of calves during the milk feeding period in a Hungarian large-scale dairy farm

Picture is for illustration only

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Keywords: Reactive Oxygen Species (ROS), free radicals, antioxidants (AO), plasma AO capacity (PAT), oxidative stress (OS), oxidative stress index (OSI)

1. SUMMARY

During the milk feeding period (from birth to weaning, generally for 60-70 days), calves receive feed milk or milk replacer rich in both protein and energy. Young animals show intense physical development and growth. Intensive oxidative metabolic processes, inadequate antioxidant defense system, oxidative stress can develop, which adversely affects the health and productivity of calves due to its cell-damaging effects. This justifies continuous monitoring of the redox status of the animals during the calf rearing period for early detection of oxidative stress. This may provide a basis for targeted antioxidant treatments to reduce calf disease-related losses.

2. Introduction

High Reactive Oxygen Species (ROS) are constantly formed in aerobic organisms, especially in the intracellular mitochondria. These radicals are required in controlled quantities for proper functioning of the body, for example in phagocytosis, apoptosis, but also in the maturation process of oocytes **[3, 12, 23, 29]**. However, if they are present in excessive amounts, they can damage the most important structures of living cells, such as lipids, proteins, or nucleic acids **[20, 21, 29]**. The body's antioxidant (AO) protection systems ensure control on acceptable safety concentration of ROS. Primary AO protection is provided by various enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidases (GPx), while secondary AOs are usually microelements (such as Copper, Iron, Zinc, Selenium), vitamins and provitamins (e.g. Vitamin E, Vitamin C, carotenoids) and other substances with AO function (e.g. albumin, flavonoids, uric acid, bilirubin, etc.) necessary for the proper operation of primary AOs **[11, 23, 29]**. When the amount of ROS in the body exceeds the ROS-elimination capacity of AO systems, so-called oxidative stress (OS) develops **[27]**.

In the calves, with the onset of own respiratory at birth, OS may develop **[14, 22, 25]**. In this case, the AO up-taken from colostrum plays an important role in the protection against OS **[1, 15]**. After birth, the amount of AO usually decreases and only increases over time, with the complete functionality of young animals' own AO defense system **[8, 30]**.

Three factors can cause OS to develop. At first, when the body suffers from a lack of energy and tries to compensate by mobilizing the body reserves. In association with lipolysis, the amount of free fatty acids in the blood increases, which can serve as a substrate for lipid peroxidation. At the same time, energy production in mitochondria may also be intensified, which may lead to an increase in the amount of ROS in the blood. Together, these two factors predispose to OS development. Third predisposing factor is depleted AO system with reduced functionality **[2, 16, 29]**. Hence, OS can develop when the body is exposed to metabolic stress for example in early lactation **[6, 24]**. Furthermore, in weaning period, the feeding of animals changes significantly, and this change also can cause metabolic stress, when the animal is characterized by a negative energy balance **[7, 30]**.

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Intense growth in young animals, due to the body's high protein demand and in parallel intensive energy production in cells, OS may develop with high probability. This is indicated by the fact that advanced protein oxidation products (AOPP) are often shown young animals at higher concentrations while the rate of AOPP/albumin (ALB) is decreasing **[8]**.

During the milking period (from birth to weaning, usually from 60 to 70 days), calves receive feed milk or milk replacers rich in both, proteins and energy. Young animals show an intensive physical development and growth. Due to the intensive oxidative metabolic processes, an improper AO defense system, OS may develop, which, due to its cell-damaging effect, adversely affects the health and productivity of calves. This justify the continuous monitoring of the redox status of animals during the calf rearing period for the early detection of OS. This can provide a basis for targeted AO treatments to reduce losses associated with calf diseases **[2, 9, 10, 13, 18, 19, 28]**.

3. Material and method

The aim of the study was to determine through monitoring of redox status, that are the ROS and AOs in equilibrium during the milk feeding period, or else OS may be considered under the ordinary feeding and housing conditions. The link between some biochemical (albumine (ALB), total protein (TProt), blood urea nintrogen (BUN), glucose (GLU), beta-hydroxybutirate (BHB), aspartate-transaminase (AST)) and OS parameters were examined too.

The tests were performed on a large-scale dairy cattle farm in Hungary. The study included 26 clinically healthy, female Holstein-Friesian calves delivered through a normal calving. The animals were individually housed in a straw bedded 147 x 109 x 117 cm Calf-Tel® Compact plastic calf houses (Calf-Tel, Germantown, Wisconsin, U.S.), which included a 109 x 320 cm fenced unroofed pen. Calves receive ad libitum milk replacer formula with 22% raw protein (Rosalac Red,Schils B. V., GM Sittard, Netherlands) following the intake of colostrum .

Blood samples were collected from the calves 4 times at a similar time after morning feeding session from the jugular vein, in week 1, 2, 3 and 6. The samples were refrigerated and transported to the lab, where the Free Radical Analytical Sytem 4 Evolvo (FRFAS4, H&D s.r.l., Parma, Italy) was used to measure the amount of reactive oxygen metabolites (dROM) and plasma AO capacity (PAT), from which the oxidative stress index (OSI) was calculated using the formula dROM/PAT x 100. From the samples, we measured some parameters of protein metabolism (albumine, (ALB), total protein (TProt) and urea (BUN)), some parameters of energy metabolism (glucose (GLU), beta-hydroxybutyrate (BHB)) and the parameter of liver health (Aspartate Amino Transferase (AST) enzyme activity). We determine the statistical distribution of redox status indicators (dROM, PAT and OSI) and tested the relationship with the metabolic parameters described above. The data was stored in Microsoft® Excel (Microsoft Corporation, Redmond, USA). For analysis version 3.1. R statistical program was used (R Core Team, 2018).

Table 1: means of dROM, PAT and OSI in each sampling

Parameter	**Sampling 1 3 - 8 days (n = 22)**	**Sampling 2 14 – 16 days (n = 26)**	**Sampling 3 21 – 25 days (n = 26)**	**Sampling 4 42 – 74 days (n = 26)**
dROM[U.Carr] mean (sd)	113 (21)	117 (17)	114 (26)	120 (42)
PAT [U.Cor] mean (sd)	2689 (316)	2439 (333)	2264 (165)	2822 (222)
OSI mean (sd)	4.24 (0.93)	4.85 (0.82)	5.03 (1.08)	4.29 (1.53)

Table 2: The difference in menas of dROM, PAT and OSI per sampling (ANOVA Tukey)

Samplings	**dROM p-value**	**PAT p-value**	**OSI p-value**
1st – 2nd	0.952	0.00871	0.2507
1st – 3rd	0.998	<0.001	0.0801
1st – 4th	0.782	0.31327	0.9982
2nd – 4th	0.973	<0.001	0.2945
2nd – 3rd	0.983	0.08872	0.9375
3rd – 4th	0.853	<0.001	0.0939

4. Results

The averages and standard deviation values of the OS monitoring parameters are presented in ***Table 1***. The mean dROM and OSI per sampling did not show a statistical difference ($p>0.05$). For PAT, there was a significant difference between the mean values per sampling ($p<0.05$), except for the first and fourth and second and third samples. The results are presented in ***Table 2.*** PAT values showed a downward trend during the first three samples and rose again only at the fourth sampling and exceeded the value of the first week.

The correlation test among observed redox- and metabolic parameters also was performed, the correlation matrix is shown in ***Table 3***.

5. Discussion

The mean of dROM values was the lowest at the first sampling and the highest at the fourth (113 sd 21 and 120 sd 42). Previously, it has been observed that the concentration of hydroperoxides was lower in the first 3 to 7 days of life than at birth, but rose again at 2nd to 3rd week of age [14]. In our study, the mean of dROM values (U.Carr 113 sd 21 and 114 sd 26) was similar in the first and third sampling, a slight increase was detected in the second (U.Car 117 sd 17) and at the fourth was the peak (U.Carr 120 sd 2), however the difference was not significant. Others also found dROM levels to be relatively stable during this period **[25]**. The dROM test can measure the amount of organic hydroperoxides **[4]**. Conjugate organic hydroperoxides are the primary products of peroxidation of polyunsaturated fatty acids **[17]**. Inconsistent results suggest that, maybe not the lipids are the primary substrates for peroxidation in this period, rather the proteins are that, and therefore, it is likely that the measurement of biomarkers of protein peroxidation products (e.g. AOPP) would be more appropriate for the detection of OS at this age.

The PAT averages showed a downward trend in the first three samples, then we recorded a significant increase in the fourth sampling, although the average values of the fourth sample were not statistically different from the first (2689 sd 316 and 2822 sd 222; $p = 0.311327$), so we can say that after the decrease in the second and third weeks, the amount of AOs returned from the sixth week to the first level following colostrum uptake. The difference between the second and third samples was trending, but not significant (2439 sd 333 and 2264 sd 165; $p = 0.08872$) when comparing the averages of the other samples (1-2, 2-4 and 3-4) the difference was significant ($p<0.05$) (See in ***Table 2***). This can be explained by the calves' own AO defense system at birth is underdeveloped and develops only in a few weeks, over time.

The OSI values varied similarly to PAT compared to each sampling. The lowest (4.24 sd 0.93) was the first, the highest (5.3 sd 1.08) at the third sampling, and the fourth was lower (4.29 sd 1.53). However, in any of cases was the difference significant. The value of the OSI is determined by the dROM and PAT. Although the average dROM increased at the fourth sampling, the decrease in PAT also reversed and showed a significant increase, i.e. the amount of AO was higher, followed by OSI decreases at the fourth sampling.

Table 3: Pearson correlations of observed parameters

	AST	BHB	BUN	OSI	PAT	Albumin	dROM	Glucose	Tprot
AST	1	0.5848	-0.1294	-0.2998	0.3141	0.3793	-0.1811	- 0.2784	0.0283
P value	-	*<0.0001*	*0.1996*	*0.0024*	*0.0015*	*<0.0001*	*0.0714*	*0.0050*	*0.7801*
BHB	0.5848	1	0.1366	-0.2090	0.3142	0.2509	-0.0565	-0.3474	0.0652
P value	*<0.0001*	-	*0.1755*	*0.0369*	*0.0015*	*0.0118*	*0.5768*	*0.0004*	*0.5191*
BUN	-0.1294	0.1366	1	0.0001	0.2112	-0.0363	0.1410	-0.0190	0.1972
P value	*0.1996*	*0.1755*	-	*0.9995*	*0.0349*	*0.7200*	*0.1617*	*0.8512*	*0.0492*
OSI	-0.2998	-0.2090	0.0001	1	-0.4285	0.3140	0.8678	0.0623	0.0899
P value	*0.0024*	*0.0369*	*0.9995*	-	*<0.0001*	*0.0015*	*<0.0001*	*0.5382*	*0.3735*
PAT	0.3141	0.3142	0.2112	-0.4285	1	0.2580	0.0594	-0.1432	0.3697
P value	*0.0015*	*0.0015*	*0.0349*	*<0.0001*	-	*0.0095*	*0.5569*	*0.1552*	*0.0002*
Albumin	0.3793	0.2509	-0.0363	0.3140	0.2580	1	0.4752	-0.3249	0.4041
P value	*<0.0001*	*0.0118*	*0.7200*	*0.0015*	*0.0095*	-	*<0.0001*	*0.0010*	*<0.0001*
dROM	-0.1811	-0.0565	0.1410	0.8678	0.0594	0.4752	1	-0.0210	0.3117
P value	*0.0714*	*0.5768*	*0.1617*	*<0.0001*	*0.5569*	*<0.0001*	-	*0.8360*	*0.0016*
Glucose	-0.2784	-0.3474	-0.0190	0.0623	-0.1432	-0.3249	-0.0210	1	-0.0374
P value	*0.0050*	*0.0004*	*0.8512*	*0.5382*	*0.1552*	*0.0010*	*0.8360*	-	*0.7117*
Tprotein	0.0283	0.0652	0.1972	0.0899	0.3697	0.4041	0.3117	-0.0374	1
P value	*0.7801*	*0.5191*	*0.0492*	*0.3735*	*0.0002*	*<0.0001*	*0.0016*	*0.7117*	-

Medium or higher (r >0.4) correlation was detected only in one case, between dROM and ALB. The other metabolic parameters shown no or just low association with redox parameters in our study. The medium positive correlation (r = 0.48, p<0.0001) ***(Table 3)*** between dROM-ALB were detected may be explained by the fact that ALB is one of the most significant AO factors in the blood **[26]**. Many of its physiological and pharmacological functions are known. By its structure, it has a significant binding capacity and an important function in the transport of metals, fatty acids, cholesterol, bile pigments, hormones and pharmaceutical active substances, and in addition, it plays a key role in the regulation of osmotic conditions. AO's significant role in defense is demonstrated by the fact that more than 70% of free radical binding is related to serum ALB. Protein-bound Copper and Iron ions are less likely to participate in the Fenton reaction, forming hydroxy radicals in the presence of Cu^{2+} and Fe^{2+} H_2O_2, thus ALB contributes in the AO defense.

Based on our the results it seems, that PAT and OSI may be used for numerical expression of efficiency of AO defense systems in calves, but the quantity of lipid-hydroperoxides indicated by dROM test did not show any difference during the sampling period, as proteins are still likely to be the primary substrate for ROS. It is worth considering how biomarkers (e.g. AOPP) change in the pre-weaning period.

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7. References

[1] Abuelo A., Gandy J.C.C., Neuder L., Brester J., Sordillo L.M.M. (2016): Short communication: Markers of oxidant status and inflammation relative to the development of claw lesions associated with lameness in early lactation cows. *Journal of dairy science* **99** 5640–5648.

[2] Abuelo A., Hernández J., Benedito J., Castillo C. (2019): Redox Biology in Transition Periods of Dairy Cattle: Role in the Health of Periparturient and Neonatal Animals. *Antioxidants* **8** 20.

[3] Al-Gubory K.H., Fowler P.A., Garrel C. (2010): The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *International Journal of Biochemistry and Cell Biology* **42** 1634–1650.

[4] Alberti A., Bolognini L., Macciantelli D., Caratelli M. (2000): The radical cation of N, N-diethyl-para- phenylendiamine: a possible indicator of oxidative stress in biological samples. *Res. Chem. Intermed.* **26** 253–267.

[5] Aswegen A.S. and Van Engelbrecht A.S. (2009): The relationship between transformational leadership, integrity and an ethical climate in organisations. *SA Journal of Human Resource Management* **7**.

[6] Bernabucci U., Ronchi B., Lacetera N., Nardone A. (2005): Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. *Journal of dairy science* **88** 2017–2026.

[7] Buchet A., Belloc C., Leblanc-Maridor M., Merlot E. (2017): Effects of age and weaning conditions on blood indicators of oxidative status in pigs. *Plos One* **12** e0178487.

[8] Celi P. and Gabai G. (2015): Oxidant/Antioxidant Balance in Animal Nutrition and Health: The Role of Protein Oxidation. *Frontiers in Veterinary Science* **2** 1–13.

[9] Chen J., Lindmark-Månsson H., Gorton L., Åkesson B. (2003): Antioxidant capacity of bovine milk as assayed by spectrophotometric and amperometric methods. *International Dairy Journal* **13** 927–935.

[10] Clausen M.R., Skibsted L.H., Stagsted J. (2009): Characterization of major radical scavenger species in bovine milk through size exclusion chromatography and functional assays. *Journal of Agricultural and Food Chemistry* **57** 2912–2919.

[11] Descalzo A. M.and Sancho A. M. (2008): A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. *Meat Science* **79** 423–436.

[12] Frandson R.D., Wilke W.L., Fails A.D. (2013): *Anatomy and Physiology of Farm Animals*, 7th edn. A John Wiley & Sons, Inc., Publication.

[13] Friel J.K., Martin S.M., Langdon M., Herzberg G.R., Buettner G.R. (2002): Milk from mothers of both premature and full-term infants provides better antioxidant protection than does infant formula. *Pediatric Research* **51** 612–618.

[14] Gaál T., Ribiczeyné-Szabó P., Stadler K., Jakus J., Reiczigel J., Kövér P., Mézes M., Sümeghy L. (2006): Free radicals, lipid peroxidation and the antioxidant system in the blood of cows and newborn calves around calving. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology* **143** 391–396.

[15] Goff J.P., Horst R.L. (1997): Physiological Changes at Parturition and Their Relationship to Metabolic Disorders. *Journal of Dairy Science* **80** 1260–1268.

[16] Kehrli M.E., Nonnecke B.J., Roth J.A. (1989): Alterations in bovine lymphocyte function during the periparturient period AJVR 1989 50 215 Kehrli.pdf. *American journal of veterinary research* **50** 207–214.

[17] Lee J., Koo N., Min D.B. (2004): Reactive oxygen species, aging, and antioxidative nutraceuticals. *Comprehensive Reviews in Food Science and Food Safety* **3** 21–33.

[18] Lindmark-Månsson H. and Åkesson B. (2000): Antioxidative factors in milk. *British Journal of Nutrition* **84** 103–110.

[19] McGrath J.J. (2016): Accelerated pre-weaning growth rates in dairy calves: do antioxidants have a place? *Animal Production Science* **56** 1275.

[20] Miller J.K., Brzezinska-Slebodzinska E., Madsen F.C. (1993): Oxidative stress, antioxidants, and animal function. *Journal of dairy science* **76** 2812–2823.

[21] Mitchell P.D. (1961): Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* **191** 144–148.

[22] Mutinati M., Pantaleo M., Roncetti M., Piccinno M., Rizzo A., Sciorsci R.L. (2014): Review Article Oxidative Stress in Neonatology . A Review Oxidative Stress in Human Neonatology. *Reprod Dom Anim* **49** 7–16.

[23] Palmieri B. and Sblendorio V. (2007): Oxidative stress tests: Overview on reliability and use. Part II. *European Review for Medical and Pharmacological Sciences* **11** 383–399.

[24] Pedernera M., Celi P., García S.C., Salvin H.E., Barchia I., Fulkerson W.J. (2010): Effect of diet, energy balance and milk production on oxidative stress in early-lactating dairy cows grazing pasture. *Veterinary journal (London, England : 1997)* **186** 352–7.

[25] Ranade R., Talukder S., Muscatello G., Celi P. (2014): Assessment of oxidative stress biomarkers in exhaled breath condensate and blood of dairy heifer calves from birth to weaning. *Veterinary journal (London, England : 1997)* **202** 583–587.

[26] Roche M., Rondeau P., Singh N.R., Tarnus E., Bourdon E. (2008): The antioxidant properties of serum albumin. *FEBS Letters* **582** 1783–1787.

[27] Sies H., Beckmann R., Brigelius R., Cadenas E., Clark I.A., Cohen G.C., Cowden W.B., Evans C.G., Flohé L., Frei B., Früstenberger G., Giertz H., Hamers M.N., Jones D.P., Kappus H., Loschen G., Marks F., Orrenius S., Reed D.J., Richter C., Roos D., Schulte-Frohlinde D., Smith M.T., Stern A., Thor H., Sonntag C. von Wills E.D., Willson R.L. 1985 *Oxidative Stress*. Academic Press Inc., London.

[28] Soberon M.A., Liu R.H., Cherney D.J.R. (2012): Short communication: Antioxidant activity of calf milk replacers. *Journal of Dairy Science* **95** 2703–2706.

[29] Sordillo L.M. and Aitken S.L. (2009): Impact of oxidative stress on the health and immune function of dairy cattle. *Veterinary Immunology and Immunopathology* **128** 104–109.

[30] Yin J., Wu M.M., Xiao H., Ren W.K., Duan J.L., Yang G., Li T.J., Yin Y.L. (2014): Development of an antioxidant system after early weaning in piglets. *Journal of Animal Science* **92** 612–619.