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Relationship between parity and oxidative stress in high-performance Polish Holstein-Friesian cows after the peak of lactation

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Abstract: *Relationship between parity and oxidative stress in high-performance Polish Holstein-Friesian cows after the peak of lactation.* There are many factors, which may expose cows attacks of free radicals. The highest level of oxidative stress appears in the parturition period and at the peak of lactation. Therefore, the purpose of this study was to demonstrate the relationship between the parity and oxidative stress in high-performance Polish Holstein-Friesian (PHF) cows after the peak of lactation. Seventy PHF cows were selected for the experiment according to: age (35 primiparous and 35 multiparous in the second lactation) and stage of lactation (after the peak of lactation; at days 61–90 in milk). Samples of milk and blood were collected in monthly intervals, from 61st–90th till about 271st day of lactation. Study results demonstrated a significant impact of the parity and days in milk of cows on the formation of oxidative stress markers. The primiparous cows were characterized by lower levels of GluRed and Gpx. The lowest level of oxidative stress was observed in the months after the peak to about 250 days of lactation. Based on study results, it can be concluded that that younger animals were more exposed to free radicals and oxidative stress.

Key words: oxidative enzymes, dairy cows, Holstein-Friesian breed, milk, blood, parity

INTRODUCTION

The total antioxidant capacity is the body's ability to remove excess of oxygen free radicals, understood as the sum of all the antioxidant elements in the organism. The organism has many antioxidant systems that are important in preventing oxidative stress. Free radicals may be neutralized by some active substances, such as: vitamin C, vitamin E, uric acid, bilirubin, thiols and glutathione (Crujeiras et al. 2008, Puppel et al. 2015).

There are two main groups of antioxidants: endogenous and exogenous ones. The endogenous antioxidants are produced in natural reactions in the organism. Most of them are antioxidant enzymes, which are the first line of body defense against oxidative stress, like e.g.: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px-E), and glutathione reductase (GluRed). Selected enzymes reduce or break down free radicals (Chiumiento et al. 2006, Puppel et al. 2015). Unfortunately, it is difficult to correctly determine levels of

antioxidants for cows, because of their different needs depending on the stage of lactation, and also because of the lack of external symptoms. Therefore, it is good to know levels of oxidative stress induced at each stage of the whole lactation period (Gaál et al. 2006, Pedernera et al. 2010, Celi 2011). The antioxidant potential decreased during physiological changes, and this may be an indirect cause of inflammation or mastitis (Stefanon et al. 2005, Sordillo and Aitken 2009). Because of the strain on the body's metabolism, high-performance cows are more exposed to oxidative stress. In this situation, the probability of metabolic diseases development increases and problems may appear with reproduction. Even with proper feeding of cows, the "metabolic depletion" of the animal may induce oxidative stress (Lykkesfeldt and Svendsen 2007, Celi 2011, Puppel et al. 2015).

It has already been proved that the highest level of oxidative stress appears in the parturition period or the peak of lactation (Bernabucci et al. 2005, Pintea et al. 2006, Kapusta et al. 2018). It can be considered as an organism effort to adapt to higher reactive oxygen species (ROS) production. Considering also cows welfare, it is however important to know the antioxidants capacity in the latest stage of lactation. Researchers have shown that the level of anti-oxidative enzymes decreases at the beginning of lactation. After the peak of lactation, it slowly returns to normal in natural conditions (Castillo et al. 2006, Pintea et al. 2006, Celi et al. 2010). The age of cows seems to be an interesting aspect in this respect. Some researchers reported that younger cows, especially the primiparous ones, were more exposed to oxidative stress. Lactation is something new to those animals, and connected with a lot of negative factors, e.g. stress caused by the milking process, effort caused by milk production or calving (Piccione et al. 2007).

Therefore, the purpose of this study was to demonstrate the relationship between the parity and oxidative stress in high-performance Polish Holstein-Friesian cows after the peak of lactation.

MATERIAL AND METHODS

The study was carried out at the experimental dairy farm of the Warsaw University of Life Sciences – SGGW (WULS-SGGW). Cows were kept in a free-stall dairy shed and fed a total mixed ration (TMR) *ad libitum*. The ingredient composition of TMR was (kg/day): maize silage – 21; alfalfa silage – 9.50; corn silage – 5.0; soybean meal – 2.10; pasture ground chalk – 0.1; vitamin mix – 0.16; rapeseed meal – 2.20; and magnesium oxide – 0.05. While, its chemical composition (g/kg DM) was as follows: ash – 50; crude protein – 85; acid detergent fiber – 223; and neutral detergent fibre – 354.

Seventy Polish Holstein-Friesian cows were selected for the experiment according to: parity (35 primiparous and 35 multiparous in the second lactation), and stage of lactation (after the peak of lactation; at days 61–90 in milk).

The cows were milked daily at 07:00 and 18:30 using a milking parlor and milk meters. The combined milk from the morning and afternoon milking was a representative sample for analysis.

The milk was placed in sterile bottles (250 mL) and immediately transported to the Cattle Breeding Division for analysis.

Blood samples (10 mL) were taken from each cow by jugular venipuncture into a heparinized tube, separated by centrifugation at room temperature and transported to the WULS-SGGW laboratory.

Samples of milk and blood were collected in monthly intervals (VIII samplings): sampling I – cows were between day 61 and 90 of lactation; sampling II – cows were between day 91 and 120 of lactation; sampling III – cows were between day 121 and 150 of lactation; sampling IV – cows were between day 151 and 180 of lactation; sampling V – cows were between day 181 and 210 of lactation; sampling VI – cows were between day 211 and 240 of lactation; sampling VII – cows were between day 241 and 270 of lactation; and sampling VIII – cows were beyond day 271 of lactation.

Milk gross composition including contents of: fat, total protein and lactose, was determined using a Milko-Scan FT--120 analyzer (Foss Electric, Hillerod, Denmark).

Malondialdehyde (MDA) concentration in milk was determined using a NanoQuant Infinite M200 Pro analyzer (Tecan Austria GmbH, Grödig, Austria) at a wavelength of 532 nm according to the methodology described by Kapusta et al. (2018).

Concentrations of GluRed, GPx, SOD, TAS in blood plasma (TASp) and TAS in milk (TASm) were established using a NanoQuant Infinietie M200Pro analyzer (Tecan Austria GmbH, Grödig, Austria) with a dedicated ELISA Kit, according to the methodology described by RANDOX (Randox Laboratories, Crumlin, United Kingdom).

The obtained data were analyzed statistically by two-way ANOVA, and Tukey post-hoc test using SPSS23 software. Data were presented as least squares means with standard error of the mean. Only the interactions between factors whose influence was statistically significant ($P \leq 0.05$) were considered. The correlations were determined by the Pearson coefficient.

The statistical model was:

$$Y_{ijkl} = \mu + A_i + B_j + (A_i \cdot B_j) + e_{ijk}$$

where:

Y_{ijkl} – dependent variable;
μ – overall mean;
A_i – fixed effect of the parity (i = 1, 2);
B_j – fixed effect of stage of lactation (j = 1–7);
$(A_i \cdot B_j)$ – interaction between parity and stage of lactation;
e_{ijkl} – residual error.

RESULTS AND DISCUSSION

The study showed that milk from primiparous cows contained more fat, and less protein and lactose during the whole lactation period, compared to milk of the multiparous cows (Table 1). In addition, it demonstrated a direct relationship between malondialdehyde (MDA) level and fat content; i.e. the higher level of fat was associated with a higher level of MDA. A similar correlation was demonstrated by Kapusta et al. (2018). In turn, Pedernera et al. (2010) reported that factors associated with a high level of

TABLE 1. Milk content regarding lactation stage of primiparous (PC) and multiparous (MC) cows

Days in lactation		Fat (%)		Protein (%)		Lactose (%)	
		PC	MC	PC	MC	PC	MC
61–90	*LSM*	4.36^{ABCDEF}	2.69^{ABCDEF}	$3.06^{ABCDEFG}$	$3.12^{ABCDEFG}$	4.95^{ABCDEF}	5.15^{ABCDEF}
	SEM	0.797	0.335	0.216	0.258	0.120	0.142
91–120	*LSM*	3.41^{GHIJKL}	3.07^{GHIJKL}	$3.31^{AHIJKLM}$	$3.32^{AHIJKLM}$	4.99^{GHIJKl}	5.10^{aGHIJK}
	SEM	0.321	0.288	0.310	0.267	0.143	0.119
121–150	*LSM*	4.28^{AGMnop}	3.74^{AGMnop}	3.79^{BH}	3.55^{BH}	4.81^{Al}	5.07^{aA}
	SEM	0.352	0.815	0.675	0.176	0.391	0.138
151–180	*LSM*	4.39^{BH}	4.16^{BH}	3.73^{CI}	3.57^{CI}	4.85^{BG}	5.01^{BG}
	SEM	0.889	0.748	0.377	0.2441	0.124	0.169
181–210	*LSM*	4.80^{CIn}	4.28^{CIa}	3.74^{DJ}	3.56^{DJ}	4.78^{CH}	4.99^{CH}
	SEM	0.252	0.703	0.435	0.222	0.145	0.144
211–240	*LSM*	4.72^{DJo}	4.37^{DJo}	3.60^{EKn}	3.54^{EKn}	4.75^{DI}	5.01^{DI}
	SEM	0.986	0.612	0.330	0.224	0.162	0.140
241–270	*LSM*	4.81^{EKM}	4.49^{EKM}	3.77^{FL}	3.69^{FL}	4.68^{EJ}	5.01^{EJ}
	SEM	0.984	0.634	0.306	0.242	0.290	0.106
271–305	*LSM*	4.58^{FLp}	4.44^{FLp}	3.87^{GMn}	3.74^{GMn}	4.80^{FK}	4.98^{FK}
	SEM	0.673	0.662	0.193	0.328	0.118	0.136

Means in the same column marked with the same letters differ significantly at: lowercase – $P \leq 0.05$; capitals – $P \leq 0.01$.

oxidative stress were severely negative energy balance and lower levels of milk production. Results of our study enable concluding that that younger animals were more exposed to free radicals and oxidative stress.

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) represent the main forms of the intracellular antioxidant defense (Puppel et al. 2015). Tüzün et al. (2002) showed that GPx may be considered as an indicator of oxidative stress. Additionally, Bernabucci et al. (2005) reported that an increase of plasma GPx activity might reflect an altered oxidative status in the pre- and post-calving periods. The activity of GluRed was at a similar level during the first 200 days of lactation in both analyzed groups of cows. In the case of primiparous cows, the study showed a decrease in the activity of both Gpx and GluRed after 210 days of lactation. Additionally, levels of antioxidant enzymes (GluRed and Gpx) were lower in blood plasma of the primiparous compared with the multiparous cows over the entire lactation period (Table 2). Konvičná et al. (2015) demonstrated a lower concentration of Gpx in the final stage of lactation, which was also confirmed in our study. The reported differences between the analyzed groups of cows might have induced an imbalance between production of reactive oxygen metabolites and reduction of antioxidants, as well as

TABLE 2. Antioxidant capacity of blood plasma and milk of primiparous (PC) and multiparous (MC) cows

Days in lactation		MDA (nM/mL)		GluRed (U/L)		Gpx (U/L)		SOD (U/L)		TASp (mmol/L)		TASm (mmol/L)	
		PC	MC	PC	MC	PC	MC	PC	MC	PC	MC	PC	MC
61–90	*LSM*	28.01^{a}	30.14^{a}	80.22^{A}	84.16^{A}	332.12^{a}	508.58^{a}	245.93	220.98	2.14	1.22	1.21^{abC}	0.96^{abC}
	SEM	0.459	0.401	0.010	0.803	0.085	0.251	0.877	0.172	0.013	0.017	0.054	0.035
91–120	*LSM*	34.65	32.63	87.63^{a}	85.83^{b}	324.67^{bcEF}	464.10^{bcAB}	284.09	242.20	2.23	1.35	1.04^{d}	1.20^{d}
	SEM	0.394	0.467	0.298	0.466	0.507	0.817	0.436	0.334	0.042	0.020	0.017	0.060
121–150	*LSM*	30.95	31.07	82.59^{b}	87.32^{c}	393.52^{d}	505.90^{d}	260.94	255.42	2.18	1.36	1.06^{e}	1.24^{e}
	SEM	0.412	0.693	0.634	0.9948	0.290	0.112	0.601	0.239	0.071	0.071	0.033	0.043
151–180	*LSM*	32.19	30.85	85.17	94.65	451.65^{b}	540.16^{b}	231.89	258.00	2.10	1.51	1.05	1.30
	SEM	0.973	0.355	0.645	0.416	0.935	0.498	0.284	0.526	0.082	0.083	0.004	0.019
181–210	*LSM*	33.52^{a}	34.20^{a}	88.90	100.86	493.01^{adE}	599.01^{adA}	235.82	249.56	2.22	1.37	1.10^{a}	1.38^{a}
	SEM	0.639	0.110	0.433	0.129	0.845	0.473	0.837	0.596	0.053	0.082	0.093	0.027
211–240	*LSM*	28.43	34.84	86.67	102.19	411.43^{F}	600.57^{B}	249.35	250.81	1.94	1.29	1.25^{Cde}	1.46^{dCe}
	SEM	0.879	0.458	0.545	0.501	0.736	0.516	0.006	0.375	0.040	0.051	0.054	0.075
241–270	*LSM*	28.35	33.18	80.74	98.82	395.01	555.63	246.05	256.59	1.80	1.42	1.14^{b}	1.41^{b}
	SEM	0.442	0.649	0.850	0.598	0.535	0.287	0.283	0.352	0.029	0.037	0.026	0.045
271–305	*LSM*	29.64	31.11	80.13^{abA}	95.39^{Abc}	336.20^{c}	586.32^{c}	241.79	262.98	2.03	1.33	1.18	1.32
	SEM	0.631	0.313	0.448	0.122	0.516	0.806	0.688	0.199	0.066	0.055	0.096	0.035

MDA – malondialdehyde; GluRed – glutathione reductase; Gpx – glutathione peroxidase; SOD – superoxide dismutase, TASp – total antioxidant status in blood, TASm – total antioxidant status in milk.

Means in the same column marked with the same letters differ significantly at: lowercase – $P \leq 0.05$; capitals – $P \leq 0.01$.

lipid peroxidation process. Based on the obtained results, it can be concluded that that younger animals were more exposed to free radicals and oxidative stress.

Concentration of MDA was at a similar level in both groups (Table 2). Its highest value was determined in primiparous milk after 90 days in lactation, immediately after the peak of lactation. Also Bernabucci et al. (2005) demonstrated that cows after calving showed a decrease of plasma and erythrocyte SOD, and an increase of MDA. In addition, as demonstrated by the study, the lower level of MDA was related with a higher concentration of antioxidant enzymes. Our results were also consistent with findings reported by Castillo et al. (2005).

The study demonstrated that the total antioxidant status (TAS) in blood plasma of primiparous cows between 90th and 210th day of lactation was at a similar level. However, in the later period, a significantly decrease has been reported in the antioxidant capacity, probably due to reduction in the supply of exogenous antioxidants. In the case of multiparous cow, we showed that TAS plasma was at a similar level during the whole analyzed lactation period. Its lower level was determined only in the third month probably because of the post-natal period. The same tendencis was found in TAS measured in milk. Castillo et al. (2003) compared the level of TAS in high (35 L/day) and low (20 L/day) yielding cows, and demonstrated small differences between these groups.

The study showed a significant correlation between MDA concentrations and individual components (Table 3). As shown by study results, the high concentrations of MDA was associated with lowered level: TASs, TASm, GluRed and Gpx.

CONCLUSION

The study has demonstrated that the age of cows plays an important role in oxidative stress induction. In all cases, indicators of oxidative stress were higher in the plasma of primiparous

TABLE 3. Pearson correlations

	GluRed	Gpx	SOD	TASp	TASm	MDA
GluRed	1	0.420**	NS	0.750**	–0.402**	–0.315**
Gpx	0.420**	1	–0.221**	NS	–0.220**	–0.380**
SOD	NS	–0.221**	1	–0.342**	–0.597**	0.618**
TASp	0.750**	NS	–0.342**	1	–0.312**	–0.520**
TASm	–0.402**	–0.220**	–0.597**	–0.312**	1	–0.420**
MDA	–0.315**	–0.380**	0.618**	–0.520**	–0.420**	1

GluRed – glutathione reductase; Gpx – glutathione peroxidase; SOD – superoxide dismutase; MDA – malondialdehyde; TASm – total antioxidant status determined in milk; TASp – Total antioxidant status determined in blood plasma.

** The correlation significant at the 0.01 level (two-sided); * The correlation significant at the 0.05 level (two-sided); NS – not significant.

than multiparous cows. In both groups, oxidative stress has increased slightly in the last months of lactation compared to the previous months. The lowest level of oxidative stress was observed in the months after the peak (after 90 days) to about 250 days of lactation. Oxidative homeostasis was stable between 90th and 250th day of lactation.

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Streszczenie: *Zależność między wiekiem a stresem oksydacyjnym u wysokowydajnych krów rasy polskiej holsztyńsko-fryzyjskiej po szczycie laktacji.* Zdolność antyoksydacyjna to zdolność organizmu do usuwania reaktywnych form tlenu, które powodują stres oksydacyjny w organizmie. Istnieje wiele czynników, które mogą narazić krowy na ataki wolnych rodników. Najwyższy poziom stresu oksydacyjnego pojawia się w okresie porodu i w szczycie laktacji. Celem tego doświadczenia było wykazanie związku między wiekiem a poziomem stresu oksydacyjnym u wysokowydajnych krów rasy polskiej holsztyńsko-fryzyjskiej (PHF) po szczycie laktacji. Do eksperymentu wybrano 70 krów PHF według: wieku (35 pierwiastek i 35 wieloródek w drugiej laktacji) i fazy laktacji (po szczycie laktacji, między 61. a 90. dniem laktacji). Próbki mleka i krwi pobierano w miesięcznych odstępach, od 61.–90. do średnio 271. dnia laktacji. Badania wykazały istotny wpływ wieku krów i fazy laktacji na kształtowanie się markerów stresu oksydacyjnego. Pierwiastki charakteryzowały się niższym poziomem GluRed i Gpx. Najniższy poziom stresu oksydacyjnego wykazano w miesiącach po szczycie i do około 250. dnia laktacji. Na podstawie uzyskanych wyników można stwierdzić, że młodsze zwierzęta były bardziej narażone na działanie wolnych rodników i stres oksydacyjny.

Słowa kluczowe: enzymy oksydacyjne, krowa mleczna, rasa holsztyńsko-fryzyjska, mleko, krew, wiek

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