Influence of management and genetic merit for milk yield on the oxidative status of plasma in heifers

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Abstract

This study was part of a larger study that addresses whether milk production levels affect health risks in dairy cows taking into account the effects of genotype, environment and interactions between these. Plasma samples were collected from 80 Holstein Friesian heifers at 2 weeks pre-partum and at 4 and 8 weeks post-partum in a balanced 2x2x2 factorial design with the factors breeding value for milk production (high or low), milk frequency (2 or 3 times a day) and feed energy density (high or low). The following parameters indicative of the oxidative status were measured by spectrophotometric methods: ferric reducing antioxidant power (FRAP), glutathion peroxidase activity (GSH-Px) and two measures of lipid oxidation, namely malondialdehyde (MDA) concentration and paraoxonase activity (PON). Significant effects occurred only for FRAP and GSH-Px. FRAP, i.e. a measure for the total antioxidant capacity of plasma, was lower before calving than 4 and 8 weeks after calving ($P<0.001$), and was lower in the high vs. the low energy density feed group ($P<0.05$). The plasma GSH-Px activity was higher 4 weeks after calving compared with 2 weeks before calving ($P<0.05$). These results indicate changes in the plasma oxidative status of heifers around parturition but minimal influences of genetic merit for milk yield, feed energy density, milking frequency and, consequently, milk production level per se.

Introduction

Over the last decades, intensive genetic selection, improved nutrition and changed management have significantly increased the milk yield of dairy cows. These economically favourable developments have some drawbacks, such as an increase in negative energy balance (NEB) during early lactation, an increase in the incidence of metabolic diseases and a reduction in reproductive performance (Van Arendonk et al., 1989; Pryce et al., 1999). For the health of dairy cows, the peripartum and early lactation periods are especially critical, because of the drastic physiological changes (Goff & Horst, 1997). During this transition period, cows seem to be more sensitive to oxidative stress (Miller & Madsen, 1994; Ronchi et al., 2000), which may contribute to periparturient metabolic diseases (Miller et al., 1993; Brzezinska-Slebodzinska et al., 1994). Oxidative stress in a living organism results from an imbalance between the production of reactive oxygen metabolites (ROM) and the capacity of the antioxidant mechanism to neutralise these ROM (Sies, 1991). In the last few years, the detection of damage caused by free radicals and protection against it has become increasingly important in clinical medicine as a complementary tool in the evaluation of the metabolic status (Castillo et al., 2003).

This study focuses on oxidative stress and assesses if milk production level modifies the risk for health problems in dairy cattle, taking into account that milk production reflects genotype, environment and interactions between these two. Therefore, different parameters related to the oxidative status of plasma from periparturient dairy cows were evaluated.

Material and methods

Experimental set-up

Samples were derived from a study described in detail by Beerda et al. (accepted). The experiment was a 2x2x2 balanced factorial design with breeding value for milk production (high or low), milk frequency (2 or 3 times a day) and feed energy density (high or low) as factors. Holstein Friesian heifers were selected from Dutch dairy farms based on the Dutch expected breeding value for 'INET' and a minimum of 87.5% Holstein-Friesian pedigree. INET is the Dutch production index for milk, fat and protein. Average INET expected breeding values of the low and the high breeding value group were -25 (ranging from -117 to 64) and 177 (ranging from 133 to 241) respectively. The heifers were kept in 4 adjacent sections of a cubicle house with concrete slatted floors and monitored individually for feed intakes. Water and Partially Mixed Ration (PMR) were available ad libitum. Concentrates were provided via feeding boxes and in the milking parlor. Cows fed high energy density rations had access to a higher quality PMR (consisting of maize silage, 49% dry matter (DM) of PMR; grass silage, 30%; soybean meal and customized meal, 21%) and 8 kg concentrates per day. Those fed low energy density rations had access to a lower quality PMR (consisting of grass silage, 86% DM of TMR; concentrates, 14%) and received 3 kg concentrates per day. The NEL value (net energy value for lactation) of the PMR was 6.5 and 5.9 MJ/kg DM for the high and the low energy density ration respectively. The concentrate NEL value was 7.5 and 7.4 MJ/kg DM for the high and the low energy density ration respectively. Rations only slightly differed for the Dutch feed protein evaluation values DVE and OEB.

Plasma analyses

Blood samples from 100 heifers were collected by puncture in the jugular vein at various time points. Samples were centrifuged (10 minutes; 1500 g) and plasma was stored at –20°C until analysis. Samples from 80 animals (balanced across the three experimental factors) at 2 weeks prepartum and at 4 and 8 weeks postpartum were used for the present study. The following parameters related to oxidative status were measured by spectrofotometric methods: ferric reducing antioxidant power (FRAP), glutathion peroxidase activity (GSH-Px), superoxide dismutase activity (SOD), TBARS concentration and paraoxonase activity.

The FRAP method is a measure of the total anti-oxidative capacity of plasma (Benzie & Strain, 1996). The method is based on the conversion of Fe^{3+}-tripyridyltriazine (TPTZ) to Fe^{2+}- TPTZ by antioxidants present in the plasma with the formation of a blue colour. The absorbance is measured at 593 nm and 37°C for 20 minutes. Using a calibration curve, FRAP values are calculated and expressed in µmol Fe^{2+} formed per L plasma.

Glutathion peroxidase (GSH-Px) is an endogenous enzyme involved in the reduction of H_2O_2 and hydroperoxides. The activity is determined by measuring the oxidation of NADPH (340 nm, 37°C) in the presence of reduced glutathion and H_2O_2 (De Vore & Greene, 1982), and is expressed as units (U) with one unit equivalent to the amount of enzyme that is needed to oxidise 1 µmol NADPH per min per mL plasma. Superoxide dismutase (SOD) converts superoxide radicals ($O_2^{\bullet -}$) into H_2O_2. The activity is measured as the inhibition of the oxidation of NADPH in the presence of superoxide radicals (Paoletti & Mocali, 1990).

However, in the present study no SOD activity in the plasma samples could be detected, and this parameter is not further discussed.
The concentration of MDA (malondialdehyde) and paraoxonase activity are both indicators of lipid oxidative stability. The concentration of MDA is determined by the TBARS (thiobarbituric acid reactive substances) method, wherein the absorbance at 532 nm is measured of a coloured complex that is formed from the reaction of MDA with 2-TBA in acid environment. It is expressed in nmol MDA per mL plasma. Paraoxonase is a high-density lipoprotein associated enzyme that protects against the oxidation of lipoproteins. Following the addition of paraoxon, the formation of p-nitrophenol is measured kinetically at 405 nm and 37°C (Turk et al., 2004). The activity is expressed as nmol p-nitrophenol produced per min per L plasma.

Additional data and statistics
Milk yield and other metabolic traits were collected in the larger study (Beerda et al., accepted for milk yield traits and energy balance, in preparation for metabolic traits). At 4 and 8 weeks postpartum, milk yield (kg/day), energy corrected milk yield (ECM) (kg/day), milk protein yield (g/day), milk fat yield (g/day), milk lactose yield (g/day) were assessed. At 2 weeks prepartum and at 4 and 8 weeks postpartum, the plasma concentration of non-esterified fatty acids (NEFA, mmol/L), beta-hydroxybutyrate (BHBA, mmol/L), insuline (pmol/L) and glucose (mmol/l) was determined. The energy balance (MJ /day) was also assessed.
Statistical analysis was performed using the Linear Mixed Models procedure of SPSS for windows version 12.0. The mixed model included the fixed effects of sampling week, breeding value, milk frequency and feed energy level, and animal as random effect. Only the significant two-way interaction terms between the fixed effects were kept in the final models. Comparison of means was performed using Bonferonni as post-hoc test.

Results and Discussion

Effect of genetic merit and management factors on plasma oxidative status
For the oxidative status traits, none of the interaction terms was significant. The total antioxidant capacity of plasma, expressed as FRAP-values, was lower at 2 weeks before calving than at 4 and 8 weeks after calving ($P<0.001$) (Table 1). A decrease in total antioxidant capacity with approaching parturition was also reported by Brzezinska et al. (1994) and by Chawla and Kaur (2004). This could be due to metabolic and endocrine adjustments related to the metabolism of the foetus and the mammary gland (Bernabucci et al., 2005) and/or due to feed factors. Mean FRAP-values were also significantly lower in the high compared to the low feed energy level group (Table 1), which might be ascribed to a higher PUFA and pro-oxidant content and/or a higher metabolic rate on the high energy feed. No effect of breeding value and milk frequency was observed on the FRAP-values ($P>0.05$). However, it should be mentioned that according to the data of Chawla and Kaur (2004) and Castillo et al. (2005), measures of total antioxidant capacity of plasma particularly alter in the last week before and the first week after parturition. Hence, our sampling time points probably did not allow to detect changes occurring in the early transition period, and the effects of management and genetic factors thereon.
The GSH-Px activity of plasma showed higher values 4 weeks after calving compared with those measured 2 weeks before calving ($P<0.05$) (Table 1). Also Bernabucci et al. (2005) observed higher plasma GSH-Px values after calving compared with data registered before calving. This increase of plasma GSH-Px activity can be considered as an indirect indicator of

oxidative stress (Tüzün et al., 2002). Breeding value, milk frequency and feed energy level did not induce significant differences in the plasma GSH-Px activity (Table 1).
Mean paraoxonase and MDA levels were unaffected by time and the experimental factors in this study. Both measurements can be considered as a reflection of lipid oxidation and apparently this does not play an important role here. Again, sampling time might be critical. Turk et al. (2004) reported a lower paraoxonase activity in cows in the early postpartum period (10-15 days after calving) compared to cows in late lactation (14-16 weeks after calving). Castillo et al. (2005) found an increase in MDA values one week before and one week after calving compared to earlier and later time points, however with wide individual variations. On the other hand, Bernabucci et al. (2005) observed an increase in TBARS values at 5 days before calving and up to 30 days after calving compared with samples taken from 30 to 10 days before calving.

Table 1. Mean values of oxidative status markers in dairy cow plasma according to management factors and genetic value for milk yield

	Week peripartum			Breeding value		Milk frequency		Feed energy level		RMSE
	-2	4	8	Low	High	2 x	3 x	Low	High	
FRAP (µmol Fe^{2+} per L)										
Mean	213[a]	238[b]	248[b]	235	231	230	236	237[a]	228[b]	27.7
P		< 0.001		0.276		0.131		0.021		
GSH-Px (U)										
Mean	0.139[a]	0.152[b]	0.146[ab]	0.146	0.145	0.146	0.145	0.144	0.147	0.030
P		0.028		0.843		0.760		0.466		
TBARS (nmol MDA per mL)										
Mean	1.41	1.29	1.11	1.27	1.28	1.27	1.27	1.19	1.35	0.677
P		0.118		0.922		0.980		0.237		
Paraoxonase (nmol p-nitrophenol produced per min per L)										
Mean	10.03	9.68	10.61	10.67	9.55	9.92	10.30	9.46	10.75	7.71
P		0.744		0.276		0.709		0.209		

[1] U = amount of enzyme needed to oxidise 1 µmol NADPH per min per mL
[a,b] Means with a different superscript are significantly different (P<0.05)

No relationship between oxidative status parameters and milk yield or metabolic traits
Milk yield and metabolic traits are only briefly discussed here. Milk yield expressed as FPCM and milk fat was significantly (P<0.05) lower at 8 weeks postpartum than at 4 weeks postpartum. As expected, all milk yield traits were significantly higher in the groups with the highest breeding value (all P<0.05), the 3 times milking frequency (all P<0.01) and the high caloric density feed (all P<0.001). One or more interaction terms were significant for most traits, but these are not further discussed here. The calculated energy balance was more negative at 4 weeks vs. 8 weeks postpartum and metabolic traits were significantly (P<0.001) affected in the same direction, including insuline NEFA and BHBZ. Caloric density had the strongest effect on metabolic traits with low caloric density being associated with significant (P<0.01) increases in metabolic stress. The clear effects of the experimental factors on milk yield and metabolic traits in the present study were thus not accompanied by changes in the oxidative status traits. This was also apparent from the correlation coefficients. Except for a

significant positive relationship between plasma MDA and glucose levels (r=0.31), and a negative relationship between FRAP values and insuline and glucose levels (r=-0.27 and r=-0.20 respectively), no relationships between oxidative status traits and performance or metabolic traits were noticed in the present study.

Conclusions

The results of this study indicate minimal influences of genetic merit for milk yield, feed quality, milking frequency and interactions between these on anti-oxidant capacity in plasma of heifers. Possibly, the latter represents a homeostatic system, like for example blood pH, which is controlled tightly in comparison to systems that are more allostatic in nature. There are no clear indications that the anti-oxidant capacity is impaired by high milk yield.

References

BEERDA, B., OUWELTJES, W., SEBEK, L.B., WINDIG, J.J. & VEERKAMP, R.F. Effects of genotype by environment interactions on milk yield, protein and energy balances. Journal of Dairy Science (accepted).

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.

BRZEZINSKA-SLEBODZINSKA, E., MILLER, J.K., QUIGLEY, J.D. & MOORE, J.R. (1994). Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. Journal of Dairy Science, 77, 3087-3095.

BENZIE, I.F.F., STRAIN, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "oxidant power": the FRAP assay. Analytical Biochemistry 239, 70-76.

CASTILLO, C., HERNANDEZ, J., LOPEZ-ALONSO, M., MIRANDA, M. & BENEDITO, J.L., (2003). Values of plasma lipid hydroperoxides and total antioxidant status in healthy dairy cows: preliminary observations. Archives of Animal Breeding, 46, 227-233.

CASTILLO, C., HERNANDEZ, J., BRAVO, A., LOPEZ-ALONSO, M., PEREIRA, V. & BENEDITO, J.L. (2005). Oxidative status during late pregnancy and early lactation in dairy cows. The Veterinary Journal, 169, 286-292.

DE VORE, V.R., GREENE, B.E., 1982. Glutathione peroxidase in post-rigor bovine semi tendinosus muscle. Journal of Food Science 47, 1406-1409.

GOFF, J.P. & HORST, R.L. (1997). Physiological changes at parturition and their relationship to metabolic disorders. Journal of Dairy Science, 80, 1260-1268.

MILLER, J.K., BRZEZINSKA-SLEBODZINSKA, E. & MADSEN, F.C. (1993). Oxidative stress, antioxidants and animal function. Journal of Dairy Science, 76, 2812-2823.

MILLER, J.K. & MADSEN, F.C. (1994). Transition metals, oxidative status, and animal health: do alterations in plasma fast-acting antioxidants lead to disease in livestock? Biotechnology in the feed industry. Pages 283-301 in Proceedings of Altech's 10th Annual Symposium T. P. Lyons and K.A. Jacques, ed. Nottingham University Press, Nottingham, UK.

PAOLETTI, F. & MOCALI, A. (1990). Determination of superoxide dismutase activity by purely chemical system based on NAD(P)H oxidation. Methods in Enzymology, 186, 209-219.

PRYCE, J.E., VEERKAMP, R.F., THOMPSON, R., HILL, W.G. & SIMM, G. (1997). Genetic aspects of common health disorders and measures of fertility in Holstein Friesian dairy cattle. Animal Science, 65, 353-360.

RONCHI, B., BERNABUCCI, U., LACETERA, N. & NARDONE, A. (2000). Oxidative and metabolic status of high yielding dairy cows in different nutritional conditions during the transition period. Proc. 51st Annual Meeting EAAP, Vienna, p. 125.

SIES, H. (1991). Oxidative Stress: Oxidants and Antioxidants. Academic Press, San Diego, CA.

TÜZÜN, A., ERDIL, A., INAL, V., AYDM, A., BAGCI, S., YESILOVA, Z., SAYAL, A., KARAEREN, N. & DAGALP, K. (2002). Oxidative stress and antioxidant capacity in patient with inflammatory bowel disease. Clinical Biochemistry, 35, 569-572.

TURK, R., JURETIC, D., GERES, D., TURK, N., REKIC, B., SIMEON-RUDOLF, V. & SVETINA, A. (2004). Serum paraoxonase activity and lipid parameters in the early postpartum period of dairy cows. Research in Veterinary Science, 76, 57-61.

VAN ARENDONK, J.A.M., HOVENIER, R. & DE BOER, W. (1989). Phenotypic and genetic association between fertility and production in dairy cows. Livestock Production Science, 21, 1-12.