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The relationship between early stages of lactation and antioxidant capacity of milk and blood plasma of PHF cows*

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The aim of this study was to evaluate the antioxidant capacity of milk and plasma, by determining the contents of enzymatic and non-enzymatic antioxidants in relation to the initial phase of lactation. The experiment was carried out at the experimental dairy farm of the Warsaw University of Life Science. Samples of milk and blood were collected from 100 cows (multiparous) for laboratory analyses in the following repetitions (4 samplings): sampling 1 – cows were between day 6 and 8 of lactation; sampling 2 – cows were between day 9 and 28 of lactation; sampling 3 – cows were between day 29 and 49 of lactation; and sampling 4 – cows were between day 50 and 70 of lactation. The recorded lLower superoxide dismutase (SOD) activity in blood of the ruminants during the postpartum period shows that animals may have experienced a higher degree of oxidative stress. At the lowest level of SOD (≤ 200 U/L), such enzymes as glutathione peroxidase (Gpx), glutathione reductase (Glu Red) and plasma total antioxidant status (TAS) showed the highest activity: 448.48 U/L, 64.61 U/L and 0.66 U/L, respectively. Additionally, an increased level of free radicals has a negative effect on the body's mineral balance in cattle, with PO_4 being the most sensitive mineral. Analyses confirm the impact of oxidative stress on metabolic or hormonal diseases in high-yielding

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cows as well as decreasing antioxidant capacity of milk. It may be concluded that among the analysed minerals calcium is sensitive to superoxide radical, which activates SOD.

KEY WORDS: antioxidant / milk / blood plasma / cow / SOD

The metabolic redox status may have important implications to cattle health and production. Oxidative stress is a consequence of an imbalance between oxidants and biological ability to quickly detoxify the reactive intermediates or repair damage in the body cells. This can, however, cause permanent tissue damage or even apoptosis [Sies 1997, Celi 2011, Puppel *et al.* 2015]. Oxidative stress is induced by reactive oxygen species (ROS), which are highly reactive, unstable chemical particles (atoms or groups of atoms) produced during reactions as a response to factors promoting free radicals: UV radiation, cigarette smoke, air pollution and stress. However, free radicals can also be produced naturally, as by-products of natural metabolic reactions in cells, such as e.g. respiratory chain and immunity cellular response (respiratory burst). During respiratory burst cells release large amounts of ROS in contact with a pathogen. The reason for high reactivity of ROS is connected with the atom of oxygen with one single electron (free radical), which readily enters into the reaction sequence. It is not consumed during reactions and can bind with new molecules or atoms. The most reactive ROS are superoxide anion ($O2^{-\bullet}$), hydroxyl radical ($OH^{\bullet}$), superoxide radical ($ROO^{\bullet}$) and alkoxy radical ($RO^{\bullet}$). ROS include also components which are not free radicals, e.g. hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), hypobromous acid (HOBr), without a single electron [Ball 2001, Celi 2011, Puppel *et al.* 2015]. However, ROS do not always have negative effects. It should be noted that at homeostasis ROS act as metabolism mediators and play an important role in redox signaling reactions.

Antioxidants are defined as chemical compounds found at very low concentrations in the organism, which stop or delay oxidation reactions in cells. The main role of antioxidants is to prevent the disturbance of homeostasis, called an oxidative stress, by neutralizing ROS and free radicals, interrupting radical reactions or capturing ROS to form stable conformation [Ball 2001, Puppel *et al.* 2015].

Organisms have also developed a mechanism to reduce oxidative stress by synthesising natural compounds which break down or remove ROS. Endogenous antioxidants are divided into enzymatic and non-enzymatic. The enzymatic mechanism of ROS defense includes superoxide dismutase, glutathione peroxidase and glutathione.

Therefore, the objective of this study was to evaluate the antioxidant capacity of milk and plasma by determining the contents of enzymatic and non-enzymatic antioxidants in relation to early stages of lactation.

Material and methods

The experiment was carried out at the experimental dairy farm of the Warsaw University of Life Sciences (WULS). The cows were kept in a free-stall barn and fed a total mixed ration (TMR) diet with the following composition (kg d^{-1}): maize silage

– 26.0, alfalfa silage – 11.30, corn silage – 4.0, soybean meal – 2.10, pasture ground chalk – 0.10, vitamin mix – 0.16 and rapeseed meal – 2.50. The chemical composition of the TMR (g kg^{-1} DM) was calculated from the chemical composition of individual dietary constituents: ash – 66, crude protein – 98, acid detergent fibre (ADF) – 235, and neutral detergent fibre (NDF) – 362.

Samples of milk and blood for laboratory analyses were collected from 100 cows (multiparous in the 2nd lactation) in the following repetitions (4 samplings): sampling 1 – cows were between day 6 and 8 of lactation; sampling 2 – cows were between day 9 and 28 of lactation; sampling 3 – cows were between day 29 and 49 of lactation; and sampling 4 – cows were between day 50 and 70 of lactation. The samples were taken on the same day from all the cows.

The cows were milked daily at 05:30 and 17:30 and milk yield was recorded at each milking. The milk was placed in sterile bottles, preserved with Mlekostat CC and immediately transported to the Cattle Breeding Division (Milk Testing Laboratory of WULS) for the analysis of composition.

Blood samples (10 mL) were taken from each cow by jugular venipuncture (by a veterinary doctor) into a heparinized tube, separated by centrifugation at room temperature (1,800×g, 15 min), and immediately transported to the Veterinary Centre of WULS for the analysis of blood biochemical parameters.

Chemical analyses

The following parameters were determined in milk**:** gross composition, fatty acids, whey proteins, fat-soluble vitamins, Ca^{+}, Mg^{+}, PO_4 and TAS.

Non-esterified fatty acids (NEFAs), β-hydroxybutyric acid (BHBA) and glucose were determined using a Biochemical BS800M analyser (PZ Cormay, Warsaw, Poland).

The gross composition of milk, i.e. contents of fat, protein, casein, and lactose, was determined by automated infrared analysis with a Milkoscan FT – 120 analyser (Foss Electric, Hillerød, Denmark).

Fatty acid methylation was performed according to the trans esterification method (EN ISO 5509). Identification of individual fatty acids in crude fat was conducted using an Agilent 7890A GC (Agilent, Waldbronn, Germany) with a flame-ionization detector, the HP Chem software and a Varian Select FAME column (100 m length, 0.25 mm diameter, 0.25 μm film thickness; Varian/Agilent Technologies, Waldbronn, Germany). The analysis involved a programmed run with temperature ramps under the conditions and temperatures described by Puppel *et al.* [2016]. Each peak was identified using pure methyl ester standards: PUFA no. 1, Lot LB 75066; PUFA no. 2, Lot LB 83491; FAME Mix RM-6, Lot LB 68242; and Supelco 37 Comp. FAME Mix, Lot LB 68887 (Supelco, Bellefonte, PA, USA).

The content of whey proteins was determined using an Agilent 1100 Series reverse phase high-performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) according to the methodology described by Puppel *et al.* [2016]. The identification of peaks as lactoferrin, lysozyme was confirmed by comparison with

the respective standards: Lf Lot 081M7021V; Lys Lot BCBD9010V (Sigma-Aldrich, St Louis, MO, USA). Separations were performed at ambient temperature using a solvent gradient on a Jupiter column C18 300A (Phenomenex, Torrance, CA, USA).

Contents of fat-soluble vitamins: β-carotene (BK) and α-tocopherol, were determined using an Agilent 1100 Series reverse phase high-performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) and a Zorbax Eclipse XDB C8 column (4.6 x 150 mm, 5 µm film thickness) according to the method described by Puppel *et al.* [2016].

Contents of Ca^+ and Mg^+ and PO_4 were assayed using REFLEKTOQUANT (Merck, Darmstadt, Germany) and test strips dedicated to their analyses (Merck, Darmstadt, Germany).

The following parameters were determined in blood plasma: SOD, GPx, GluRed and TAS. The levels of oxidative stress and concentrations of antioxidative enzymes in the samples were determined using commercially available RANDOX kits: RANSOD for superoxide dismutase (SOD), RANSEL for glutathione peroxidase (GPx), RANDOX GLUT RED for glutathione reductase (GluRed), and RANDOX TAS for the total antioxidant status (TAS). Analyses were conducted in a Nano Quant M200 PRO analyser using ELISA plates.

Statistical analysis

Statistical analysis was performed using SPSS 23 (Statistical Package for the Social Sciences) software. Data are expressed as the mean, standard variation, and multiple comparisons. Only the interactions between factors which influence was statistically significant ($p \leq 0.05$) were considered. The correlations were determined by the Pearson option.

The statistical model was:

$$Y_{ijkl} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk,}$$

where:

Y_{ijkl} – dependent variable;

μ – overall mean;

A_i – phase of lactation effect (i=1, 2, 3,4);

B_j – SOD level effect (j=1, 2, 3,);

$(AB)_{ij}$ – interaction between the lactation effect and the SOD level;

e_{ijk} – residual error.

Results and discussion

Pregnancy in dairy cows induces oxidative stress that can be a significant underlying factor leading to dysfunctional host immune and inflammatory responses, potentially increasing the incidence and severity of infectious diseases [Sordillo 2013].

The dissonance between dry and lactating cows might be related to antioxidant contents in their diets. Wachter *et al.* [1999] indicated that forage intake is positively associated with plasma antioxidant activity. Studies have shown that in the early days of lactation the level of antioxidative enzymes is much lower than in the peripartal period (Tab. 1). This is obviously associated with cows experiencing the oxidative stress at the beginning of lactation due to many factors, such as calving, resumption of milking after the dry period or postpartum diseases. The peripartal period is one of the most stressful periods in the life of dairy cows. In peripartal cows tissues consume more oxygen through normal cellular respiration during times of increased metabolic demand in order to provide the energy needed for the onset of lactation [Gitto *et al.* 2002].

Many authors have investigated relationships between the physiological changes during parturition and losses in overall antioxidant potential in dairy cows [Gitto *et al.* 2002, Bernabucci *et al.* 2005, Sordillo *et al.* 2007]. The antioxidant defense system has many components and a deficiency in any of these components can cause a reduction in the overall antioxidant status of an individual animal. In our experiment the highest level of antioxidative enzymes was reported between days 50 and 70 of lactation (Tab. 1). Values obtained between day 50 and day 70 of lactation for Glu Red, Gpx and TAS were highly significant compared with the previous stages (1st, 2nd and 3rd stage). Selenium glutathione peroxidase is an enzyme containing selenium as an essential component that confers protective effects against oxidative damage. It should be stressed here that determination of plasma cytosolic glutathione peroxidase (GPX1) activity is often used as a diagnostic tool when assessing the Se status of dairy cows [Sordillo and Aitken 2009].

No significant differences between weeks of lactation were detected only in the level of SOD, although the dependency on its concentration was the same as in the case of other enzymes (Tab. 1). On the other hand, differences were observed in the total antioxidant status in milk. The highest level of TAS was found in milk in the first days of lactation, while in the following days it declined (Tab. 1). The same relationship as for TAS was observed in NEFA, Lz and Mg contents (Tab. 3 and 4). Konvičná *et al.* [2015] reported that mean SOD activity gradually increased from 51.03 to 65.87 μkat/gHb, while GSH-Px activity decreased (in the first week after calving). Additionally, Mudron *et al.* [1999] reported that oxidation of NEFA in the liver increased the production of reactive oxygen species which directly caused the oxidative stress.

A different relationship was found for glucose, CLA, C18:1c9, β-LG, Ca, PO_4 and BK. Their concentrations were the highest in the 2nd stage of lactation (between 9-28 days) and it gradually decreased with time (Tab. 2 and 3). The lowest concentrations of these elements were found in the 3rd stage (29-49 days), except for minerals, which were the least abundant in the last stage, in contrast to antioxidative enzymes (Tab. 2 and 3). Nozière *et al.* [2006] reported that low plasma concentrations of β-carotene are connected with an increased incidence of udder infection. Cows suffering from severe mastitis tend to produce milk containing less β-carotene and more retinol than non-infected cows.

Table 1. Changes in antioxidants enzymes content in first 10 weeks of lactation

Phase of lactation	Glu Red (U/L)		Gpx (U/L)		SOD (U/L)		TAS plasma (U/L)		TAS milk (U/L)	
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM
≤8 days	42.95^{A}	4.100	267.88^{a}	77.577	231.40	47.453	0.18^{A}	0.202	1.97^{aAB}	0.680
9-28	56.24^{B}	21.073	341.41^{b}	100.581	236.26	87.204	0.32^{B}	0.160	1.58^{aC}	0.329
29-49	61.18^{C}	35.813	373.21	213.489	226.44	71.203	0.53^{C}	0.594	1.53^{AD}	0.612
50-70	82.78^{ABC}	24.655	444.48^{ab}	225.366	238.92	72.356	1.45^{ABC}	1.467	1.08^{BCD}	0.448

Glu Red – glutathione reductase, Gpx – glutathione peroxidase, SOD – superoxide dismutase, TAS – total antioxidant status.
$^{aA\cdots}$Within columns means bearing the same superscripts differ significantly at: small letters – P≤0.05; capitals P≤0.01.

Table 2. Changes in metabolites content in first 10 weeks of lactation

Phase of lactation	NEFA (mmol/L)		BHBA (mmol/L)		Glucose (mg/L)		CLAc9t11 (g/100 g of fat)		C18:1c9 (g/100 g of fat)	
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM
≤ 8 days	0.56^{ABC}	0.161	0.66^{a}	0.184	62.97	9.319	0.42^{A}	0.100	24.92^{a}	3.607
9-28	0.28^{ADE}	0.222	0.65^{A}	0.261	66.37^{Aa}	6.417	0.51^{ABC}	0.088	25.52^{AB}	2.665
29-49	0.15^{BD}	0.089	$0.94a^{Ab}$	0.606	60.49^{Ab}	6.540	0.42^{Ba}	0.071	22.05^{aA}	3.738
50-70	0.16^{CE}	0.122	0.79^{b}	0.232	63.40^{ab}	6.152	0.45^{Ca}	0.090	22.72^{B}	3.903

NEFA – non - esterified fatty acids, BHBA – β – hydroxyl butyrate acid, CLAc9t11 – conjugated linoleic acid cis9 trans11, C18:1c9 – oleic acid.
$^{aA\cdots}$Within columns means bearing the same superscripts differ significantly at: small letters – P≤0.05; capitals P≤0.01.

Only BHBA and vitamin E showed the highest concentrations between days 29 and 70 of lactation, i.e. at the peak of the lactation. A sub-optimal antioxidant status in dairy cows with the fatty liver disease implicates susceptibility to ROS. Surveys of clinical cases of hepatic lipidosis show that plasma vitamin E is consistently lower in afflicted cows than in healthy cows [Hidiroglou and Hartin 1982]. Cows with hepatic lipidosis have lower plasma vitamin A and E levels at

Table 3. Changes in selected components content in first 10 weeks of lactation

Phase of lactation	Lz (g/L)		β-LG (g/L)		Ca (mg/L)		Mg (mg/L)		PO_4 (mg/L)		BK (mg/L)		E (mg/L)	
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM
≤ 8 days	21.09^{a}	8.003	2.64^{a}	0.789	87.67^{AB}	7.263	36.00^{AB}	1.732	10.00^{a}	6.062	0.17^{A}	0.036	0.46^{AB}	0.263
9-28	15.84	3.264	3.38^{aAB}	0.408	91.57^{CD}	7.782	33.14^{CD}	4.725	14.29^{aAB}	4.573	0.26^{ABC}	0.122	0.46^{CD}	0.334
29-49	12.60^{aA}	4.394	2.52^{A}	0.904	73.31^{AC}	10.812	23.23^{AC}	5.815	10.92^{AC}	8.041	0.18^{B}	0.089	0.64^{AC}	0.170
50-70	17.66^{A}	11.909	2.79^{B}	1.057	71.97^{BD}	11.297	21.56^{BD}	4.548	7.06^{BC}	1.790	0.17^{C}	0.085	0.63^{BD}	0.176

LZ – lysozyme, β-LG – β-lactoglobulin, Ca – calcium, Mg – magnesium, PO_4 – phosphate, BK – β-carotene, E – vitamin E.
$^{aA\cdots}$Within columns means bearing the same superscripts differ significantly at: small letters – P≤0.05; capitals P≤0.01.

calving and fail to build their stores to appropriate post-calving levels [Hidiroglou and Hartin 1982].

Studies confirm a significant role of oxidative stress in metabolic or hormonal diseases in high-yielding cows as well as decreasing milk performance, and deteriorating the cytological and technological quality of milk. SOD is the first line of defense against oxidative stress, it catalyzes the disproportionation (dismutation) of a superoxide anion radical ($O2^{-\bullet}$) to H_2O_2. ($O2^{-\bullet}$), which leads to many disorders, e.g. inflammation, ageing, ischemic disorders, tumor promotion and infectious diseases. That is why SOD is considered as the most important antioxidant enzyme [Davies 2003, Kinnula 2003, Chiumiento 2006]. Three types of SOD are found in a body: extracellular (EC-CuZnSOD), mitochondrial (MnSOD), and cytoplasmic (CuZnSOD). Only EC-CuZnSOD was found in blood plasma, body fluids, lymph, extracellular matrix, and on the surface of cells [Marklund 1984, Zelko *et al.* 2002, Gacko 2006, Hai 2012]. Researchers reported lower SOD activity in blood of ruminants during the postpartum period, which shows that animals may have experienced a higher degree of oxidative stress [Celi *et al.* 2010]. At the lowest level of SOD (≤200 U/L) such enzymes as Gpx, Glu Red and plasma TAS showed the highest activity: 448.48 U/L, 64.61 U/L and 0.66 U/L, respectively (Tab. 4). In those cases the lowest activity was recorded at the medium level of SOD (201-260 U/L) and increased slightly at a higher SOD content (>260 U/L). The greatest significant differences were observed for Gpx concentration between the groups affected by the level of SOD. In the case of plasma TAS, a relationship was also found between the last group and the first and second ones (Tab. 4).

The study also showed a significant relationship between SOD levels and the content of oleic acid (C18:1c9; OA). The content of OA increased directly proportionally to the concentration of SOD and differed highly significantly between groups ($P \leq 0.01$). The same trend was found for the concentrations of NEFA and PO_4 (Tab. 5 and 6). A high concentration of non-esterified fatty acids (NEFAs) in blood is associated with a more frequent incidence of metabolic diseases in the perinatal period [Jóźwik *et al.* 2012] and thus, with increasing levels of oxidative stress.

Organisms have developed two lines of defense against ROS and oxidative stress: complexes formed in the body (endogenous antioxidants) or delivered through nutrition (exogenous antioxidants) [Gurer 2000]. Recognizing the involvement of ROS in the pathogenesis of a multitude of diseases, biomedical scientists have devoted considerable amounts of research to understanding the phenomenon of oxidative stress (OS). Oxidant/antioxidant imbalance may be related to metabolic demand and negative energy balance in these cows, as plasma ROS are correlated with plasma β-hydroxybutyrate ($r = 0.40$) and non-esterified fatty acids [Bernabucci *et al.* 2005]. Components which have shown the largest number of highly significant correlations between other, at the level of 0.01, are Glu Red, Gpx, milk TAS, Ca, Mg, and PO_4 (Tab. 7). All the minerals showed a highly significant negative correlation with the antioxidative enzymes and plasma TAS. These results indicate that an increased level of free radicals has a negative effect on the body's mineral balance in cattle. Potassium

Table 4. Changes in antioxidants enzymes content compared to the level of SOD

Content of SOD (U/L)	Glu Red (U/L)		Gpx (U/L)		SOD (U/L)		TAS plasma (U/L)		TAS milk (U/L)	
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM
≤200	64.61	6.027	448.48^{AB}	45.594	141.79^{AB}	8.761	0.66^{a}	0.262	1.51^{Aa}	0.099
201-260	59.85	4.683	320.00^{A}	35.433	220.88^{AC}	6.808	0.56^{b}	0.204	1.77^{AB}	0.077
>260	59.96	5.328	331.26^{B}	40.306	314.92^{BC}	7.745	0.62^{ab}	0.232	1.13^{aB}	0.088

Glu Red – glutathione reductase, Gpx – glutathione peroxidase, SOD – superoxide dismutase, TAS – total antioxidant status.
aA... Within columns means bearing the same superscripts differ significantly at: small letters – P≤0.05; capitals P≤0.01.

Table 5. Changes in metabolites content compared to the level of SOD

Content of SOD (U/L)	NEFA (mmol/L)		BHBA (mmol/L)		Glucose (mg/L)		CLAc9t11 (g/100 g of fat)		C18:1c9 (g/100 g of fat)	
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM
≤200	0.25	0.028	0.88	0.075	64.06	1.357	0.48	0.019	21.98^{A}	0.792
201-260	0.26	0.022	0.67	0.058	66.20^{a}	1.055	0.44	0.015	23.53^{B}	0.615
>260	0.31	0.025	0.84	0.066	59.75^{a}	1.200	0.43	0.017	25.89^{AB}	0.700

NEFA – non - esterified fatty acids, BHBA – β-hydroxybutyric, CLAc9t11 – conjugated linoleic acid cis9 trans11, C18:1c9 – oleic acid.
aA... Within columns means bearing the same superscripts differ significantly at: small letters – P≤0.05; capitals P≤0.01.

Table 6. Changes in minerals and whey proteins content compared to the level of SOD

Content of SOD (U/L)	Lf (g/L)		LZ (g/L)		Ca (mg/L)		PO_4 (mg/L)		BK (mg/L)		E (mg/L)	
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM
≤200	0.170^{a}	0.086	18.39	2.193	86.78^{A}	2.305	9.39^{a}	0.997	0.25	0.018	0.70	0.041
201-260	0.25^{ab}	0.067	16.18	1.704	78.68	1.792	10.01^{a}	0.775	0.19	0.014	0.48	0.032
>260	0.19^{b}	0.076	15.38	1.938	79.03^{A}	2.038	12.50	0.882	0.18	0.016	0.51	0.037

LZ – lysozyme, Blg – β-lactoglobulin, Ca – calcium, Mg – magnesium, PO_4 – phosphate, BK – β-carotene, E – vitamin E
aA... Within columns means bearing the same superscripts differ significantly at: small letters – P≤0.05; capitals P≤0.01.

was found to be the most sensitive mineral, as its correlation was -0.301 with Glu Red and -0.374 with Gpx. Only Ca showed a negative correlation with SOD level. It may be concluded that among the analysed minerals calcium is sensitive to superoxide radical, which activates SOD. Ca showed a positive correlation also with the level of β-carotene, which is a strong antioxidant. For comparison, magnesium showed an unexpected negative correlation with vitamin E, but a positive one with oleic acid. All the minerals showed highly significant correlations between each other and a positive correlation with milk TAS, which is exactly opposite to TAS from plasma (Tab. 7). Of

the analysed antioxidative enzymes, SOD level was correlated only with Gpx, and the correlation was negative. These enzymes do not work at the same time; Glu Red is activated after Gpx reactions with glutathione.

Studies confirm a significant role of oxidative stress in metabolic or hormonal diseases in high-yielding cows, as well as decreasing antioxidant capacity of milk.

Table 7. Pearson correlations between individual components

Item	Glu Red	Gpx	SOD	TAS plasma	TAS milk	CLAc9tr11	C18:1c9	Ca	Mg	PO_4	BK	E
Glu Red	1	0.300**	NS	0.602**	-0.219**	NS	NS	-0.212**	-0.379**	-0.301**	NS	NS
Gpx	0.300**	1	-0.248**	NS	-0.209**	NS	NS	-0.214**	-0.214**	-0.374**	NS	NS
SOD	NS	-0.248**	1	NS	NS	NS	0.185*	-0.201**	NS	NS	NS	NS
TAS plasma	0.602**	NS	NS	1	-0.279**	NS	-0.190*	NS	-0.274**	-0.211**	NS	NS
TAS milk	-0.219**	-0.209**	NS	-0.279**	1	NS	-0.152*	0.230**	0.372**	0.229**	NS	-0.399**
CLAc9tr11	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.153*	NS
C18:1c9	NS	NS	0.185*	-0.190*	-0.152*	NS	1	0.165*	0.294**	NS	0.167*	NS
Ca	-0.212**	-0.214**	-0.201**	NS	0.230**	NS	0.165*	1	0.701**	0.422**	0.221**	NS
Mg	-0.379**	-0.214**	NS	-0.274**	0.372**	NS	0.294**	0.701**	1	0.502**	NS	-0.234**
PO_4	-0.301**	-0.374**	NS	-0.211**	0.229**	NS	NS	0.422**	0.502**	1	NS	NS
BK	NS	NS	NS	NS	NS	0.153*	0.167*	0.221**	NS	NS	1	0.306**
E	NS	NS	NS	NS	-0.399**	NS	NS	NS	-0.234**	NS	0.306**	1

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

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