



OPEN Shortening of the telomere length during the transition period of dairy cows in relation to biological stress

Manon Dewulf¹✉, Osvaldo Bogado Pascottini^{1,2}, Stijn Heirbaut³, Maya Meesters¹, Dries S. Martens⁴, Tim S. Nawrot^{4,5}, Mingqi Zhang³, X. P. Jing^{3,7}, Leen Vandaele⁶, Veerle Fievez³, Mieke Van Eetvelde¹ & Geert Opsomer¹

Telomere length (TL) is a recognized biomarker for ageing in multiple species. In dairy cattle, the transition period is considered a very stressful period. We hypothesized that TL shortens during this period. Holstein cows ($n = 61$) were followed during the transition period. Blood and milk samples were collected at $-7, 3, 6, 9, 21$ d relative to calving to determine concentrations of oxidative, energetic metabolic, and inflammatory markers. Average relative leukocyte TL was measured by a modified qPCR protocol 7 d before and 21 d after parturition. We confirmed TL attrition during the transition period ($P = 0.02$), as TL was 1.05 ± 0.229 (mean $\pm$ SD) before, and 0.97 ± 0.191 (mean $\pm$ SD) after parturition. Univariable analyses assessed associations between blood markers and TL shortening. Greater plasma oxidative parameters, including oxidized glutathione and glutathione peroxidase, were positively and negatively (respectively) associated with TL attrition. Higher blood α - and β -globulin were all positively associated, while IGF-1, albumin-globulin ratio and albumin were negatively associated with TL attrition. Greater serum amyloid A and haptoglobin were linked with greater TL shortening. This study reveals significant TL shortening during the transition period in dairy cows and identifies significant associations with oxidative stress, metabolic stress, and inflammation. While these associations are observed, no causality can be established. Our findings suggest the need for further research to explore the effects of transition-related stress on TL dynamics.

Keywords Oxidative and energetic metabolic stress, Systemic inflammation, Longevity, Telomere-attrition

The evolutionary theory states that reproduction comes at the expense of somatic maintenance and longevity. In human medicine, the number of pregnancies is related to accelerated cellular ageing in women¹. Their findings demonstrate that both mitotic and non-mitotic measurements of cellular ageing show similar associations with the number of pregnancies, indicating a potential cost of reproduction in terms of accelerated ageing.

Cellular ageing can be measured using telomere length (TL), and TL attrition correlates with increased mortality risk^{2,3}. Telomeres consist of tandemly repeated DNA nucleotides “TTAGGG”⁴, which shorten after each cell cycle⁵. When telomeres are critically short, a response is triggered leading to senescence⁶. Thus, TL has been considered a biomarker for human health, ageing, and lifespan⁷. Recently, TL has also emerged in high-yielding dairy cattle as potential marker for longevity⁸, welfare⁹, and energetic metabolic stress¹⁰.

Telomere length in cattle tends to shorten significantly in early life and remains relatively stable thereafter¹¹. This observation aligns with longitudinal studies in other species, such as humans, which also report an initial telomere attrition followed by a plateau phase in adulthood¹². However, telomere attrition is accelerated by intrinsic and external factors, such as UV-light¹³, chronic infection¹⁴, and exposure to oxidative stress¹⁵. In addition, TL change in dairy cattle appears to be influenced by environmental variables, such as weather conditions, making it a promising biomarker for the consequences of physiological stress¹⁶.

The transition period of dairy cows is defined as the period from three weeks before until three weeks after calving¹⁷. It has been characterized as a crucial stage, during which cows undergo significant metabolic and

¹Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium. ²School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland. ³Department of Animal Sciences and Aquatic Ecology, Faculty of Bioengineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium. ⁴Centre for Environmental Sciences, Hasselt University, Agoralaan Gebouw D, 3590 Diepenbeek, Belgium. ⁵Research Unit Environment and Health, Department of Public Health & Primary Care, Leuven University, Leuven, Belgium. ⁶ILVO, Scheldeweg 68, 9090 Melle, Belgium. ⁷Research Center for the Belt and Road, Lanzhou University, Lanzhou 730000, China. ✉email: manon.dewulf@ugent.be

physiological adaptations¹⁸, often resulting in metabolic stress. Increased oxygen consumption is needed to cope with the physiological stress brought on by the development of the nearly full-term calf, parturition, and the beginning of milk synthesis and secretion. This increased oxygen consumption results in the generation of free radicals (oxidants), finally leading to oxidative stress^{18,19}. Furthermore, hormonal changes such as the decrease in progesterone and the increase in estrogens, as well as immune system dysfunction around calving, make cows more susceptible to systemic inflammation, leading to a higher incidence of both metabolic as well as infectious diseases in the transition period²⁰.

Although both oxidative and energetic metabolic stress as well as systemic inflammation are significant risk factors for health disturbances during the transition period, they rarely have been studied simultaneously. In addition, results solely based on markers of stress without association to cellular damage may be difficult to interpret. In the present study, we aimed to assess TL dynamics during the transition period in relation to biological stress. We hypothesize that TL shortening occurs during the transition period in dairy cows. We aim to identify associations between TL attrition and specific blood variables related to oxidative stress, metabolic stress, and inflammation during this critical phase.

Results

Descriptives

For the determination of the TL, the first blood collection took place 7.13 ± 3.95 (mean $\pm$ SD) days before parturition. Telomere length before parturition was 1.05 ± 0.229 . The second blood collection for TL assessment took place 21.0 ± 0.70 days after parturition. Telomere length after parturition was 0.97 ± 0.191 . The sampling interval to assess the Δ -TL was on average 26.65 ± 5.28 days.

A total of 142 blood samples from the 71 animals were included for TL assessment. Six of the 142 blood samples were classified as inadequate due to excessive dilution, impeding accurate TL assessment. Furthermore, the qPCR curves of two additional samples were excessively skewed, resulting in their exclusion from the analysis. Eight cows calved prematurely, and blood samples were taken from these animals on the day of parturition. Notably, there was overlap between the inadequate samples and those from the prematurely calved cows, with 4 cows contributing to both categories. After excluding the inadequate and skewed samples, as well as those related to the premature calvings, TL data were available for 61 animals.

Only animals with successful TL assessment both before and after parturition ($n = 61$) were included in the subsequent analyses to identify factors that were associated with TL attrition. To determine energetic metabolic stress parameters, blood sampling took place 7.13 ± 3.95 days prior to calving and 3.1 ± 0.28 , 5.9 ± 0.55 , 9.1 ± 0.49 , and 21.0 ± 0.70 days after calving. Additionally, blood for oxidative and inflammatory markers, along with other metabolic stress parameters, was collected 21.0 ± 0.70 days after calving (Fig. 1).

Descriptive data (BCS, DMI, weight, and results of the blood and milk analyses) are shown in Table 1.

Factors associated with TL attrition

There was a decrease in TL of 0.08 ± 0.243 ($P = 0.02$) in samples collected before versus after parturition. Parity ranged from two to seven, but no association was observed between TL and parity ($P = 0.17$). Presence of clinical

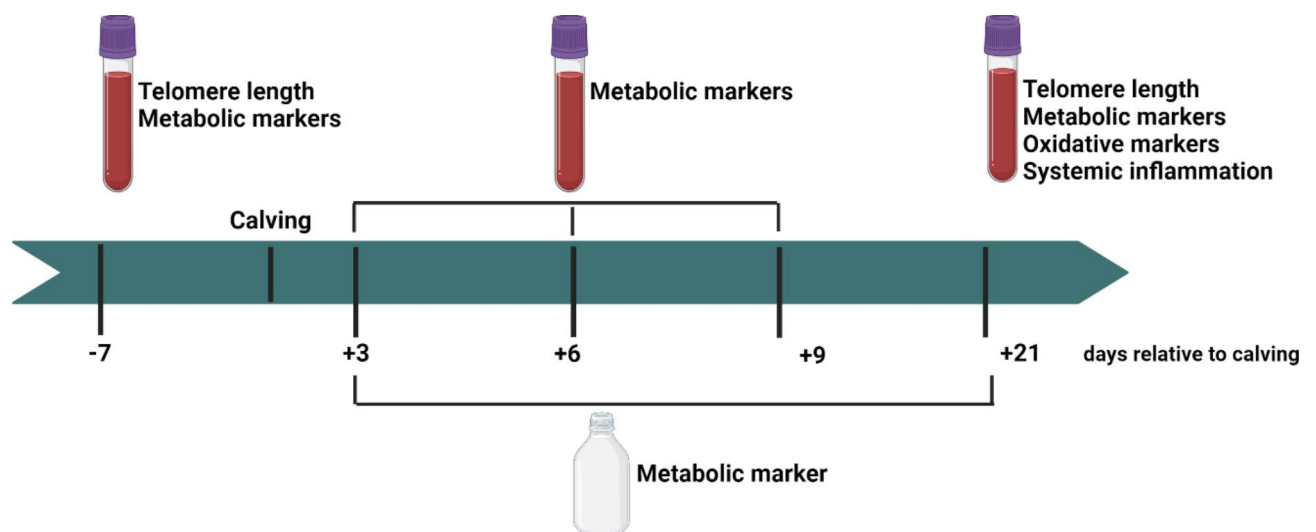


Fig. 1. Schematic overview of the experimental design, showing the selected timepoints for sample collection (-7 , 3 , 6 , 9 , 21 relative to calving). EDTA blood samples were tested for telomere length. Heparin blood samples were analysed for oxidative markers (GSSG, GPx, MDA, ORAC, SOD) and inflammation (Hp and SAA). Serum and sodium fluoride blood samples were used for the analysis of the metabolic markers (glucose, BHB, NEFA, IGF-1, insulin, fructosamine, AG ratio, Albumin, α -globulin, β -globulin, γ -globulin, Total protein, Urea, AST). Milk samples for measuring BHB and Urea were collected daily for 21 days, excluding the first two days following parturition. (Number of animals = 61).

Telomere length	Mean $\pm$ SD	
Before parturition (TLBP)	1.05 $\pm$ 0.229	
After parturition (TLPP)	0.97 $\pm$ 0.191	
Δ -TL	0.08 $\pm$ 0.242	
Cow characteristics	DTP	
Total dry matter intake (kg)	– 7	15.12 $\pm$ 2.887
	3	17.44 $\pm$ 4.336
	6	18.23 $\pm$ 4.351
	9	19.68 $\pm$ 4.716
	21	22.67 $\pm$ 3.625
Body condition score (1–5)	– 7	3.42 $\pm$ 0.297
	3	3.38 $\pm$ 0.247
	6	3.40 $\pm$ 0.253
	9	3.39 $\pm$ 0.247
	21	3.22 $\pm$ 0.258
Body weight (kg)	– 7	797.60 $\pm$ 71.304
	3	722.52 $\pm$ 71.236
	6	713.88 $\pm$ 72.662
	9	704.18 $\pm$ 69.175
	21	692.48 $\pm$ 66.887
Oxidative parameters		
GSSG (GSSG/GSH + GSSG)	21	9.16 $\pm$ 4.238
GPx (μ mol/(min·mL plasma))	21	0.055 $\pm$ 0.027
MDA (nmol/g plasma)	21	4.27 $\pm$ 0.999
ORAC (μ M Trolox eq.)	21	12.17 $\pm$ 3.167
SOD (U/mL plasma)	21	14.82 $\pm$ 13.012
Metabolic parameters (blood)		
AG ratio (A:G)	21	0.73 $\pm$ 0.153
Albumin (%)	21	41.80 $\pm$ 5.16
α -globulin (%)	21	15.95 $\pm$ 1.753
β -globulin (%)	21	8.99 $\pm$ 1.300
γ -globulin (%)	21	26.55 $\pm$ 6.994
Total protein (g/L)	21	79.54 $\pm$ 16.661
Urea (mmol/L)	21	2.81 $\pm$ 0.578
AST (IU/L)	21	87.67 $\pm$ 19.151
Inflammatory parameters		
SAA (μ g/mL)	21	41.02 $\pm$ 67.471
Hp (mg/mL)	21	0.38 $\pm$ 0.460

Table 1. Cow characteristics, telomere length, oxidative, metabolic, and inflammatory parameters along with respective units of measurement, based on days relative to parturition (PTD) (Mean $\pm$ SD) (Number of animals = 61).

disease in the transition period (N=9) was also not associated with TL (P=0.07). Table 2 shows results from the univariable analyses of the oxidative, metabolic, and inflammatory markers on day 21 post parturition. For oxidative stress parameters associated with TL attrition, GSSG% (P<0.0001) was positively associated with TL shortening, while GPx (P<0.0001) was negatively associated. The metabolic parameters, α - (P<0.0001) and β -globulin (P<0.001) were positively associated with telomere attrition, while albumin (P<0.001) was negatively associated. Similarly, the albumin-to-globulin ratio (P<0.001) was negatively associated. For systemic inflammation, SAA (P<0.0001) and Hp (P=0.002), were both positively associated with telomere attrition. The descriptives and results from the univariable models for the six energetic metabolic markers, measured in both blood and milk, are presented in Table 3. Among these markers, solely IGF-1 exhibited significant negative associations with Δ -TL on days 3 (P<0.01), 6 (P<0.1), and 21 (P<0.001) post partum (Fig. 2).

Variable (unit)	Estimate ± Std. Error	P-value	P-value*	R ^{2**}	Note
Oxidative parameters					
GSSG (GSSG/GSH + GSSG)	0.005 ± 0.0015	0.001	0.005	0.08	1
GPx (μmol/(min·mL plasma))	− 0.828 ± 0.2354	0.004	0.005	0.08	2
MDA (nmol/g plasma)	0.008 ± 0.0063	0.352	0.9250	0.06	3
ORAC (μM Trolox eq.)	− 0.0007 ± 0.00192	0.850	1.0000	0.06	4
SOD (U/mL plasma)	− 0.0001 ± 0.00047	0.666	1.0000	0.06	4
Metabolic parameters					
AG ratio (A:G)	− 0.152 ± 0.0413	0.007	0.008	0.13	5
Albumin (%)	− 0.004 ± 0.0012	0.007	0.008	0.13	6
α-globulin (%)	0.017 ± 0.0036	0.0004	0.0008	0.16	4
β-globulin (%)	0.012 ± 0.0046	0.009	0.012	0.10	7
γ-globulin (%)	0.001 ± 0.0011	0.366	1.00	0.08	6
Total protein (g/L)	0.0008 ± 0.00037	0.524	1.00	0.07	3
Urea (mmol/L)	0.013 ± 0.0102	0.609	1.00	0.06	3
AST (IU/L)	1.86 e-05 ± 3.168 e-04	0.520	1.00	0.06	4
Inflammatory parameters					
SAA (μg/mL)	2.80 e-04 ± 8.976 e-05	0.004	0.02	0.11	4
Hp (mg/mL)	0.040 ± 0.0131	0.032	0.04	0.11	8

Table 2. Univariate analysis of blood parameters related to oxidative, metabolic, and inflammatory stress on Day 21 (D21). The results shown are from the univariable model associated with the outcome variable Δ-TL (telomere length before parturition – telomere length after parturition). Disease and parity were forced into the model, while season and BCS were offered and retained when $P < 0.1$ (see Note). ($N = 61$). *P-value after multiple testing, using the Bonferroni correction (p.adjust () function). **The multiple R-squared. 1. Model covariate is BCS ($P < 0.05$). 2. Model covariate is season ($P < 0.05$). 3. Model covariates are BCS and season ($P < 0.05$ and $P < 0.01$, respectively). 4. Model covariates are BCS and season ($P < 0.05$). 5. Model covariates are BCS and season ($P < 0.005$ and $P < 0.0005$, respectively). 6. Model covariates are BCS and season ($P < 0.005$). 7. Model covariate is BCS ($P < 0.005$). 8. Model covariates are BCS and season ($P < 0.005$ and $P < 0.05$, respectively).

Variable	Unit	Days in milk				
		− 7	3	6	9	21
Blood						
Glucose	mmol/L	3.42 ± 0.297	3.24 ± 0.397	2.97 ± 0.369	2.87 ± 0.387	3.12 ± 0.497
BHB	mmol/L	0.69 ± 0.140	0.85 ± 0.281	0.94 ± 0.378	1.06 ± 0.665	0.97 ± 0.463
NEFA	mmol/L	0.18 ± 0.142	0.52 ± 0.314	0.64 ± 0.300	0.58 ± 0.268	0.55 ± 0.278
Insulin	ng/mL	1.08 ± 0.674	0.28 ± 0.239	0.26 ± 0.227	0.23 ± 0.171	0.28 ± 0.150
IGF1	ng/mL	143.49 ± 58.731	125.96 ± 57.828**	116.18 ± 53.110*	109.75 ± 53.767	115.57 ± 57.942***
Fructosamine	μmol/L	281.02 ± 49.035	261.62 ± 38.862	257.15 ± 41.345	253.58 ± 38.154	266.41 ± 44.642
Milk						
BHB	mmol/L		0.02 ± 0.034	0.09 ± 0.069	0.11 ± 0.095	0.143
Urea	mmol/L		17.00 ± 6.690	17.02 ± 7.285	16.20 ± 4.987	14.79 ± 4.762

Table 3. Summary of blood and milk energetic metabolic markers at d −7, 3, 6, 9, and 21 in lactation, including cow characteristics; presented as mean ± SD. Additionally, univariate analysis results for the outcome variable Δ-TL, with disease and parity forced into the model, while season and BCS were offered and retained when $P < 0.1$ ($N = 61$). * $P < 0.1$ ** $P < 0.01$ *** $P < 0.001$; Model covariates are season and BCS ($P < 0.01$ and $P < 0.1$, respectively).

Discussion

The cost of reproduction is established in other species^{1,2} and has been linked to telomere attrition²¹. Although the transition period has been proven to be an important period of biological stress in high-producing dairy cows^{22–24}, no link has yet been researched with TL. Therefore, we hypothesize TL shortening occurs during the transition period in dairy cows. Additionally, we aim to identify associations between TL attrition and specific blood variables related to oxidative stress, metabolic stress, and inflammation during this critical phase.

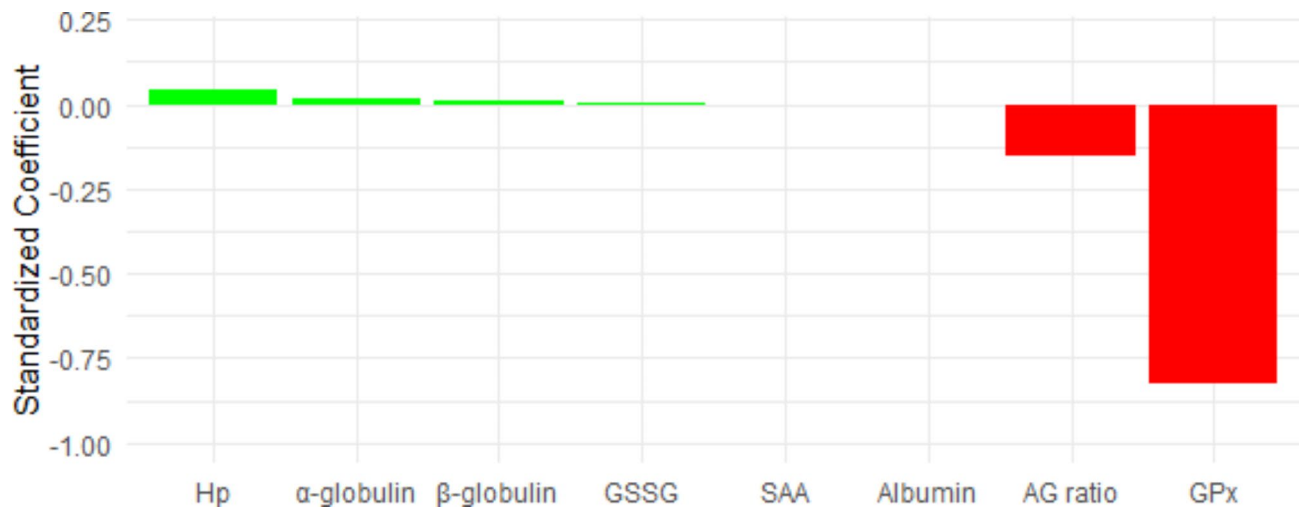


Fig. 2. Standardized regression coefficients for the association between blood parameters of oxidative stress, inflammation, and metabolic stress, and Δ -TL on day 21 post parturition (change in telomere length before and after parturition), adjusted for lactation number, health status, season, and BCS. The green color represents a positive association, while the red shows a negative association.

In the present study, TL ranged from 0.76 to 1.63 before parturition to 0.71–1.40 after parturition. Literature reports similar levels of average TL to the levels found in the current study^{11,16}. In cattle, telomere length undergoes substantial shortening during early life, followed by a period of relative stability¹¹. This pattern is consistent with longitudinal studies in other species, such as humans¹², where the rate of TL attrition in adults is typically slow, averaging less than 40 base pairs per year²⁵. Given that TL remains relatively stable in adult cows, and we observed significant TL shortening over a 26-day interval, the observed telomere shortening is likely attributable to the specific stressors of the transition period rather than a general effect of ageing. However, it would be valuable in future research to collect blood samples again during the lactation period to determine if TL recovers. This might also be an explanation as to why no significant association was found between TL and parity. Furthermore, future research could also benefit from longitudinal comparisons across different life stages to further elucidate the specific impact of various life events on telomere dynamics.

Descriptives of the stress parameters are consistent with findings from other studies on stress during the transition period^{22–24}. Our findings show that GSSG, α - and β -globulin, SAA and Hp are positively associated with TL attrition, meaning that greater blood concentrations of these markers are correlated with TL shortening. On the other hand, greater peripheral concentrations of GPx, IGF-1, albumin and the AG-ratio were negatively associated with TL shortening (=less TL attrition). To the best of our knowledge, this study is the first to research the association between TL and oxidative and metabolic parameters, as well as markers for systemic inflammation in the transition period of dairy cows.

Oxidative stress, arising from ROS production exceeding antioxidant defenses, plays a crucial role in cellular physiology^{18,19}. In the present study, we evaluated five oxidative markers. These markers profoundly impact TL and integrity across species, including humans, birds and mouse²⁶. Oxidative stress disrupts the proper structure and function of TL in multiple ways: by the accumulation of oxidized bases²⁷, by impeding the binding of shelterin proteins²⁷, and by increasing DNA double-strand breaks (DSBs) or replication stress²⁸. Prolonged oxidative culminates in cellular senescence, a state of irreversible growth arrest associated with ageing²⁸. Redox parameters like GPx and GSSG exhibit robust correlations with TL, while others like MDA and SOD lack commensurate associations²⁹. GPx and GSSG promote longevity by counteracting H_2O_2 and mitigating oxidative stress. In the present study, associations between GSSG and GPx concentrations and telomere length attrition were observed, consistent with findings reported in literature in mice and dairy cows^{29–32}.

During the transition period, high-yielding dairy cows undergo systemic inflammation, leading to the investigation of markers like serum amyloid A (SAA), haptoglobin, and the albumin-to-globulin ratio to assess inflammation and immune dysfunction³³. Systemic inflammation significantly affects cellular biology, including telomere length, through various mechanisms³⁴. The current study found a negative association between the AG ratio and telomere attrition. Comparable findings have been reported in human medicine, where the AG ratio has been suggested as a potential indicator of individual ageing³⁵. However, the mechanism linking AG ratio and TL remains unknown. Similarly, the positive association between Hp and SAA levels and telomere attrition found in this study, lacks comprehensive research and documentation in literature. In human medicine, a positive linear correlation between SAA and telomere shortening was observed, while another study in wild passerines showed no association between haptoglobin and telomere attrition^{36,37}. While the connection between stress, health, and longevity has been extensively discussed, additional research is required to fully clarify the underlying mechanisms of SAA, Hp, and the AG ratio in the regulation of TL.

Alfa- and beta-globulin were found to be positively associated with TL attrition. Both α - and β - globulins are acute-phase proteins, and their levels may increase in response to inflammation³⁸. On the other hand, higher

albumin levels are linked with a lower inflammatory state in human literature³⁹. Furthermore, another study showed that people with the highest average serum albumin values had the lowest risk of mortality and vice versa⁴⁰. Our research indicates a negative association between albumin and TL attrition, suggesting that higher levels of albumin may be associated with less TL shortening. However, research on the pathways between TL and albumin, α - and β - globulins is scarce.

We found IGF-1 to be negatively associated with TL attrition. Insulin-like growth factor-1 may counteract oxidative stress and inflammation, due to the upregulation of nitric oxide synthase by IGF-1, diminishing oxidative stress^{41,42}. Our results are in line with other research where lower concentrations of IGF-1 have been linked with accelerated cellular ageing and TL shortening^{41,42}. In human medicine, higher IGF-1 was associated with longer TL, which was suggested to be indicative of healthy ageing⁴¹. While literature provides insights into the association between IGF-1 and TL, it also acknowledges discrepancies among studies^{41,42}, indicating more research is warranted to elucidate the association between TL and IGF-1.

During the transition period of high yielding dairy cows, there is an increased demand for energy. However, limitations in dry matter intake coupled with high milk yield result in a negative energy balance (NEB)^{33,43}. In the context of fat metabolism, previous studies in human medicine have revealed a nuanced relationship with TL. Van der Spek et al. identified a positive association between telomere length and specific energetic metabolic biomarkers, including cholesterol to lipid ratios⁴⁴. Gavia-García et al. further supported this connection, highlighting the fat metabolism as a contributor to telomere attrition⁴⁵. Surprisingly, in the present study, neither NEFA, BHB nor glucose were associated with TL dynamics. Non-esterified fatty acids are known for their volatility during the transition period⁴⁶, which might explain the absence of an association with telomere attrition. However, no literature is available to explain the lack of association between telomere length and energetic metabolic biomarkers in cattle, in contrast to the apparent link observed in human medicine. Further research is needed to explore the mechanisms behind TL and energetic metabolic parameters in dairy cows.

The present study has 2 major limitations. The first is the time course around calving in which blood samples have been taken. To assess the effect of the stressful events that take place during the transition period on TL length, blood sample were taken from the cows at 7 days prior to expected calving and 21 days after calving. However, the transition period of dairy cows is defined as the period from three weeks before until three weeks after calving. Despite this reduction in time, a significant reduction in TL could be shown. The latter confirms the applied study design was appropriate to assess the effect of the transition period on TL-attrition. Second, we acknowledge that the absence of a control group, such as animals not subjected to the biological stress of the transition period or comparisons with other periods of the cows' life, is a limitation of our study. While our focus was on the transition period due to its recognized biological stress, this approach limits our ability to definitively attribute the observed telomere shortening solely to biological stress during the transition period. Future research should incorporate a control group of cows in less stressful periods or non-lactating cows to provide a more robust comparison and further validate the specific impact of transition-related stress on telomere dynamics.

This study demonstrates that TL shortens during the transition period in dairy cows and identifies significant associations with oxidative stress, metabolic stress, and inflammation. While these associations are observed, no causality can be established. Literature remains inconclusive, and the underlying mechanisms are not fully understood. Our findings suggest the need for further research to explore the effects of transition-related stress on TL dynamics. Future studies, including control groups or comparisons across different life stages, are crucial to better determine the specific impacts of transition-related stress on TL and its long-term implications for dairy cattle longevity.

Materials and methods

Animals and housing

The present longitudinal observational study was conducted at the Flemish research dairy farm of the Institute for Agriculture, Fisheries and Food (ILVO) (Melle, Belgium) from May 2019 until October 2020. The study was approved by the Ethics Committee of ILVO (Melle, Belgium) under the EC number 2018/329. Samples were taken in accordance with the relevant guidelines and regulations and all authors complied with the ARRIVE guidelines⁴⁷.

A schematic overview of the experimental design is shown in Fig. 1. The study comprised 71 Holstein cows exhibiting varying parities subsequent to calving, distributed as follows: parity 2 (n = 29), parity 3 (n = 15), parity 4 (n = 11), parity 5 (n = 2), parity 6 (n = 3), and parity 7 (n = 2). Primiparous animals were excluded from the study, as they are still undergoing growth—a stressor that could independently affect TL attrition¹¹. Lactating and non-lactating animals were housed separately in a naturally ventilated free stall barn with slatted floor. Stocking density was < 1 cow/cubicle. From imminent calving (e.g., pelvic ligament relaxation, teat filling) until d 3 after calving, cows were housed in maternity pens with straw bedding within the same building. From 3 weeks before calving, cows received a mixed ration for lactating cows supplemented with a dry cow mineral premix and on average 1 kg of balanced concentrate per cow per day. Belgian-Dutch energy and protein evaluation systems were used: requirements and supply of proteins digestible at the level of the small intestine were assessed according to the Digestible Valuable Essential (DVE)-system⁴⁸ and net energy requirements and supply according to the VEM-system⁴⁹. The basic ration for the lactating cows consisted of a mixed ration that was calculated for an average adult cow of 650 kg, producing 26 kg of fat- and protein-corrected milk (FPCM), and was based on maize silage, grass silage, pressed beet pulp, and soybean meal to balance DVE and VEM requirements. Concentrate intake started at 1.7 kg of balanced concentrates, 0.2 kg formaldehyde-treated soybean meal (Covasoy Braz., FeedValid, Zaltbommel, The Netherlands) and 0.3 kg of soybean meal at d 3 after calving. Formaldehyde-treated soybean meal was increased over a period of 7 d to 1 kg, whereas the balanced concentrate was eventually increased to a maximum of 6 kg over a period of 20 d depending on individual milk yield. Detailed information about the chemical composition and concentrate increment over time is given

in Heirbaut et al.²⁴. Individual feed intake was monitored throughout the trial using roughage intake control feeding bins (Insentec, Hokofarm Group, Emmeloord, The Netherlands), except at immediate calving. During lactation, concentrate intake was monitored at the automatic concentrate providers (Greenfeed, C-Lock Inc.; DeLaval, Tumba, Sweden). Cows had ad libitum access to water. Dam information, including parity and age were extracted from the herd databases. Cows were milked twice daily, at 05.30 and 16.30 h in a 2 × 7 herringbone milking parlor, and their milk yield (kg/d) was recorded electronically.

Body condition score (BCS) was determined using a camera from DeLaval (Tumba, Sweden)⁵⁰. The measurement was performed roughly every 2 weeks during the dry period and daily during lactation. The BCS camera output data utilized in the present study ranged from 1 to 5 with 0.1 increments. Weight measurements were acquired post-milking, using an automated weighing scale (DeLaval, Ghent, Belgium). Daily body weight assessments were conducted throughout the lactation period, and biweekly weight measurements during the dry period.

Parity refers to the number of parturitions per cow. In this study, parity is treated as an ordinal variable.

The disease definitions used, were based on reported cases that required intervention by a veterinarian or farm staff. The diseases and corresponding diagnostic procedures were as follows: hypocalcemia (clinical symptoms and confirmation through a blood calcium test), ketosis (clinical symptoms and confirmation using a Keto-Test test (Elanco, Antwerp, Belgium), left displaced abomasum (clinical examination and surgery), metritis (fetid uterine discharge), and clinical mastitis (visually aberrant milk). Cows with clinical health disorders were kept in the trial and not removed from the data set.

Blood and milk collection and laboratory analyses

Average relative telomere length

Blood samples for the assessment of TL were collected at 7 d before expected and 21 d after parturition. Venous blood samples were taken in 10 mL Vacutainer® EDTA tubes (Becton Dickinson, Erembodegem, Belgium) using an 18-gauge needle and Venoject® system (Terumo, Leuven, Belgium). Samples were taken in the morning between 09.15 and 09.45 h (around 1.5 h after feeding).

Samples were analysed at the Centre for Environmental Sciences, Hasselt University (Belgium), where an interlaboratory comparison was performed of the in-house telomere assay with a US reference lab to standardize the protocol^{51,52}. DNA from whole blood was extracted, using the QIAamp DNA Mini Kit (Qiagen, Inc., Venlo, The Netherlands). DNA quantity and purity were assessed using a Nanodrop 1000 spectrophotometer (Isogen, Life Science, Utrecht, The Netherlands). Average relative telomere length was measured using a modified singleplex quantitative PCR (qPCR) method adapted from Cawthon^{53,54}. For detailed information on DNA integrity assessment and the qPCR protocol used in telomere length determination, see Supplementary File S1.

Mathematical calculation formulas are provided by Hellemans et al.⁵⁵. The method precision is shown by coefficients of variation (CVs) of 0.46%, 0.27% and 6.73% for telomere runs, single-copy gene runs and T/S ratios, respectively. In addition, the intraclass coefficient (ICC) with 95% CI of triplicate measures (T/S ratios) was 0.995 (95% CI 0.994 to 0.997), showing a high repeatability.

Leukocyte TL in relation to a standard reference DNA (T/S ratio) was measured. The T/S ratio (also referred to as 'relative TL') is proportional to the mean TL and it will be referred to as 'TL' throughout the rest of this manuscript.

Oxidative status

Five oxidative markers were measured in the blood of the cows at 21 d after parturition: the percentage of oxidized glutathione in red blood cells (GSSG%), glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA), and oxygen radical absorbance capacity (ORAC)²². To do so, blood samples were obtained from the jugular vein using 10 mL Vacutainer® heparin tubes (Becton Dickinson, Erembodegem, Belgium), and centrifuged at 1,500 × g for 10 min. After centrifugation, 4 mL plasma was transferred into four 1.5 mL Eppendorf Safe-Lock tubes (Eppendorf Belgium N.V.- S.A., Aarschot, Belgium). Then, 300 µL of the pelleted red blood cells was transferred into an Eppendorf Safe-Lock tube and 10 µL of 1 mmol/L bathophenanthrolinedisulfonic acid disodium salt hydrate solution (stored at 4 °C for max. 1 week) was added for determining the GSSG%. Next, all the Eppendorf tubes were immediately snap frozen in liquid nitrogen and stored at -80 °C until analysis. For more detailed information on oxidative markers measured in dairy cow blood plasma at 21 days post-partum, see Supplementary File S2.

The GPx activity assay was performed on blood plasma and followed the method described by Hernández et al. (2004)⁵⁶.

The SOD assay kit 19160-1KT-F (Sigma Aldrich, St. Louis, USA) was used to determine plasma SOD activity by an indirect spectrophotometric method. The analysis was conducted following the manufacturer's protocol.

The concentration of MDA in blood plasma was assessed based on the method of Grotto et al.⁵⁷.

The ORAC assay was performed on blood⁵⁸. For this purpose, a F-bottom black, 96-well microplate (VWR, Radnor, USA) was used with Trolox (0.02 mol/L) as stock solution to build the standard curve.

The GSSG (%) analysis was performed on blood plasma in high performance liquid chromatography (HPLC; Agilent 1200 series, with auto sampler, quaternary pump, column oven and DAD detector (Agilent Technologies, Diegem, Belgium))⁵⁹.

Metabolic profile

Six energetic metabolic parameters were assessed in blood samples collected at - 7, 3, 6, 9, and 21 days relative to calving: glucose, β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), insulin, insulin-like growth factor-1 (IGF-1), and fructosamine. Milk samples for measuring BHB were collected daily for 21 days, excluding

the first two days following parturition. Albumin-to-globulin ratio (AG ratio), albumin, α -, β -, γ -globulin, total protein, urea, and aspartate amino transferase (AST) were evaluated at 21 days post partum.

Blood serum and plasma were collected in the morning, between 09.15 and 09.45 h from the coccygeal vessels, using 10 mL serum blood tubes without coagulant (BD Diagnostics, Plymouth, UK) and sodium fluoride (NaF) tubes (BD Diagnostics, Plymouth, UK), respectively. Serum samples were kept at room temperature for at least 30 min before centrifugation ($1500 \times g$ for 15 min) and NaF tubes were kept in an ice box until they were centrifuged at $1000 \times g$ for 10 min at room temperature. After centrifugation, the upper layer of the tubes (serum or plasma) was harvested and transferred into labeled Safe-Lock tubes (Eppendorf, Hamburg, Germany). All measurements were conducted on serum samples except for glucose, which was measured using NaF plasma samples. All samples were stored at -20°C , except the ones for IGF-1 determination which were stored at -80°C until analysis. Glucose, BHB, NEFA, total protein, albumin, α -globulin, β -globulin, γ -globulin, urea, and AST were all analysed using a Gallery™ Discrete Analyser (ThermoFisher Scientific, MA, USA), Randox kits (Randox Laboratories Ltd, Ibach, Switzerland), and MINICAP PROTEIN(E) 6 (Sebia, Lisses, France) by the accredited laboratory of Dierengezondheidszorg Vlaanderen (DGZ, Belgium). Serum IGF-1 concentration was measured using a commercially available Bovine IGF-1 ELISA Kit (cat. no. 201–04–0024, standard range 60 to 960 ng/mL, Shanghai Sunred Biological Technology Co., Ltd., China). The detection assay ranged from 6.0 to 1.800 ng/mL.

Fructosamine was determined using the method of Johnson et al.⁶⁰. For more details see Supplementary File S3.

Daily milk samples (30 mL) were collected in the morning (05.30) for the analysis of BHB, from d 3 until 23 post partum. Samples contained preservatives (sodium azide, maximum concentration 0.02% m/m and bronopol, maximum concentration 0.005% m/m) and were stored at 4°C . Milk BHB was determined by the accredited Qlip laboratory (Zutphen, The Netherlands), which routinely performs these analyses by means of Fourier transformed infrared spectrometry (Milkoscan FT6000, Foss Electric, Hillerød, Denmark), in the context of dairy herd improvement (DHI) analyses. Milk BHB concentration was estimated based on the mid-infrared (MIR) spectra from in-house established equations.

Systemic inflammation

Serum amyloid A (SAA) and serum haptoglobin (Hp), two inflammatory markers, were assessed in the blood of cows 21 days after parturition. Serum samples were collected from the jugular vein using 10 mL Vacutainer® heparin (Becton Dickinson, Erembodegem, Belgium). SAA and Hp were determined using the SAA assay-multispecies ELISA kit and Haptoglobin assay kit (BioRepair, Sinsheim, Germany), respectively. For inflammatory status-related parameters, we maintained the intra-assay coefficient of variation below 10%.

Statistical analyses

Statistical analyses were performed in R version 4.2.1. Data on the determination of TL variations during the transition period in dairy cows are rare, especially in relation to metabolic stress and systemic inflammation. Therefore, sample size was calculated for the detection of differences in $4.2 \pm 3.2\%$ (mean $\pm$ SD) in TL before and after parturition (significance level = 0.05 and power = 80%)⁶¹. Therefore, the study required at least 62 calving cows to determine the effect of biological stress in the transition period on TL.

The delta TL (Δ -TL) from each cow, meaning the difference between TL before versus after calving, was set as the unit of interest and was normally distributed (Shapiro–Wilkinson normality test: $W = 0.97$; p -value = 0.25). All oxidative, inflammatory, and metabolic markers were analysed for normality by the Shapiro–Wilk test and, when not normally distributed ($P < 0.05$), they were transformed (square root or log10). The association between parity and TL and, disease and TL was examined using ANOVA. The effect of oxidative – (GSSG, GPx, MDA, ORAC, and SOD), inflammatory – (SAA and Hp), and metabolic biomarkers (AG ratio, albumin, α -, β -, γ -globulin, total protein, urea, AST and BHBZ) on Δ -TL were fitted in linear regression models using the `lm()` function. The models controlled for several covariates, including parity (ordinary), presence of clinical disease in the transition period (yes vs no), body condition score at calving (≤ 3.5 or > 3.5), and season of calving (summer vs fall vs winter vs spring). For all outcomes, the baseline measurement (day – 7) was included as covariate to account pre-transition values. The impact of six energetic metabolic markers (glucose, BHBA, NEFA, insulin, IGF-1, fructosamine) were assessed using linear mixed effects regression models using the `lmer()` function. The models accounted for the same covariates, while also addressing repeated measurements (days relative to calving) within individual animals by incorporating them as a random effect (1 | Cow ID).

Model residuals were assessed using a scatterplot of the studentized residuals for homoscedasticity, linear predictor for linearity, and a Shapiro–Wilk test for normality. For all transformed and non-transformed variables, the residuals of the models were normally distributed (Shapiro–Wilk $P > 0.05$). Differences between levels of explanatory variables were assessed with the Tukey post hoc test. Results are expressed as least squares means and standard errors. Statistical significance and tendency were declared at $P < 0.05$ and $P < 0.1$, respectively.

Data availability

Datasets used in this manuscript are available from the corresponding author on reasonable request.

Received: 14 May 2024; Accepted: 8 December 2024

Published online: 30 December 2024

References

1. Ryan, C. P. et al. Reproduction predicts shorter telomeres and epigenetic age acceleration among young adult women. *Sci. Rep.* **8**, 11100 (2018).

2. Sudyka, J. Does reproduction shorten telomeres? Towards integrating individual quality with life-history strategies in telomere biology. *BioEssays* **41**, 1900095 (2019).
3. Vaiserman, A. & Krasnienkov, D. Telomere length as a marker of biological age: state-of-the-art, open issues, and future perspectives. *Front. Genet.* **11** (2021).
4. Meyne, J. et al. Distribution of non-telomeric sites of the (TTAGGG)_n telomeric sequence in vertebrate chromosomes. *Chromosoma* **99**, 3–10 (1990).
5. Blackburn, E. H. Structure and function of telomeres. *Nature* **350**, 569–573 (1991).
6. Fagagna, F. et al. A DNA damage checkpoint response in telomere-initiated senescence. *Nature* **426**, 194–198 (2003).
7. Sanders, J. L. & Newman, A. B. Telomere length in epidemiology: A biomarker of aging, age-related disease, both, or neither?. *Epidemiol. Rev.* **35**, 112–131 (2013).
8. Brown, D. E., Dechow, C. D., Liu, W. S., Harvatine, K. J. & Ott, T. L. Hot topic: Association of telomere length with age, herd, and culling in lactating Holsteins. *J. Dairy Sci.* **95**, 6384–6387 (2012).
9. Bateson, M. Cumulative stress in research animals: Telomere attrition as a biomarker in a welfare context?. *BioEssays* **38**, 201–212 (2016).
10. Laubenthal, L. et al. Short communication: Telomere lengths in different tissues of dairy cows during early and late lactation. *J. Dairy Sci.* **99**, 4881–4885 (2016).
11. Seeker, L. A. et al. Longitudinal changes in telomere length and associated genetic parameters in dairy cattle analysed using random regression models. *PLoS One* **13**, e0192864 (2018).
12. Aviv, A. & Shay, J. W. Reflections on telomere dynamics and ageing-related diseases in humans. *Philos. Trans. Roy. Soc. B Biol. Sci.* **373**, 20160436 (2018).
13. Henle, E. S. et al. Sequence-specific DNA cleavage by Fe²⁺-mediated Fenton reactions has possible biological implications. *J. Biol. Chem.* **274**, 962–971 (1999).
14. Asghar, M., Bensch, S., Tarka, M., Hansson, B. & Hasselquist, D. Maternal and genetic factors determine early life telomere length. *Proc. Roy. Soc. B Biol. Sci.* **282**, 20142263 (2015).
15. von Zglinicki, T. et al. Short telomeres in patients with vascular dementia: An indicator of low antioxidative capacity and a possible risk factor?. *Lab. Invest.* **80**, 1739–1747 (2000).
16. Seeker, L. A. et al. Telomere attrition rates are associated with weather conditions and predict productive lifespan in dairy cattle. *Sci. Rep.* **11**, 5589 (2021).
17. Drackley, J. K. Biology of dairy cows during the transition period: The final Frontier?. *J. Dairy Sci.* **82**, 2259–2273 (1999).
18. Sordillo, L. M. & Aitken, S. L. Impact of oxidative stress on the health and immune function of dairy cattle. *Vet. Immunol. Immunopathol.* **128**, 104–109 (2009).
19. Lykkesfeldt, J. & Svendsen, O. Oxidants and antioxidants in disease: Oxidative stress in farm animals. *Vet. J.* **173**, 502–511 (2007).
20. Sordillo, L. M., Contreras, G. A. & Aitken, S. L. Metabolic factors affecting the inflammatory response of periparturient dairy cows. *Anim. Health Res. Rev.* **10**, 53–63 (2009).
21. Čapková Frydrychová, R. Telomerase as a possible key to bypass reproductive cost. *Mol. Ecol.* **32**, 2134–2143 (2023).
22. Zhang, M. Q. et al. Transition cow clusters with distinctive antioxidant ability and their relation to performance and metabolic status in early lactation. *J. Dairy Sci.* **106**, 5723–5739 (2023).
23. Heirbaut, S. et al. Combination of milk variables and on-farm data as an improved diagnostic tool for metabolic status evaluation in dairy cattle during the transition period. *J. Dairy Sci.* **107**, 489–507 (2024).
24. Heirbaut, S. et al. Diagnostic milk biomarkers for predicting the metabolic health status of dairy cattle during early lactation. *J. Dairy Sci.* **106**, 690–702 (2023).
25. Chen, W. et al. Longitudinal versus Cross-sectional evaluations of leukocyte telomere length dynamics: Age-dependent telomere shortening is the rule. *J. Gerontol. Ser. A* **66A**, 312–319 (2011).
26. Armstrong, E. & Boonekamp, J. Does oxidative stress shorten telomeres in vivo? A meta-analysis. *Ageing Res. Rev.* **85**, 101854 (2023).
27. Coluzzi, E., Leone, S. & Sgura, A. Oxidative stress induces telomere dysfunction and senescence by replication fork arrest. *Cells* **8**, 19 (2019).
28. Coluzzi, E. et al. Oxidative stress induces persistent telomeric DNA damage responsible for nuclear morphology change in mammalian cells. *PLoS One* **9**, e110963 (2014).
29. Martínez de Toda, I., Vida, C., Sanz San Miguel, L. & De la Fuente, M. Function, oxidative, and inflammatory stress parameters in immune cells as predictive markers of lifespan throughout aging. *Oxid. Med. Cell. Longev.* **2019**, 1–11 (2019).
30. Salminen, L. E. & Paul, R. H. Oxidative stress and genetic markers of suboptimal antioxidant defense in the aging brain: a theoretical review. *Rev. Neurosci.* **25** (2014).
31. Mikulková, K., Kadek, R., Filipek, J. & Illek, J. Evaluation of oxidant/antioxidant status, metabolic profile and milk production in cows with metritis. *Irish Vet. J.* **73**, 8 (2020).
32. Necasova, A., Pechova, A., Bodor, R. & Masar, M. Evaluation of the glutathione concentration in whole blood of dairy Holstein cows. *Vet. Med. (Praha)* **66**, 179–188 (2021).
33. Pascottini, O. B., Leroy, J. L. M. R. & Opsomer, G. Metabolic stress in the transition period of dairy cows: Focusing on the prepartum period. *Animals* **10**, 1419 (2020).
34. Niveta, J. P. S., Kumar, M. A. & Parvathi, V. D. Telomere attrition and inflammation: The chicken and the egg story. *Egypt. J. Med. Hum. Genet.* **23**, 131 (2022).
35. Maeda, T., Oyama, J.-I., Sasaki, M., Arima, T. & Makino, N. The correlation between the clinical laboratory data and the telomere length in peripheral blood leukocytes of Japanese female patients with hypertension. *J. Nutr. Health Aging* **15**, 240–244 (2011).
36. Wong, J. Y. Y., De Vivo, I., Lin, X., Fang, S. C. & Christiani, D. C. The relationship between inflammatory biomarkers and telomere length in an occupational prospective cohort study. *PLoS One* **9**, e87348 (2014).
37. Roast, M. J. et al. Telomere length declines with age, but relates to immune function independent of age in a wild passerine. *R. Soc. Open Sci.* **9** (2022).
38. Harris, D. J. Clinical tests. In *Handbook of Avian Medicine* 77–84 (Elsevier, 2009). <https://doi.org/10.1016/B978-0-7020-2874-8.0004-3>.
39. Don, B. R. & Kaysen, G. Poor nutritional status and inflammation: Serum albumin: Relationship to inflammation and nutrition. *Semin. Dial.* **17**, 432–437 (2004).
40. Fuls, M., Stout, R. L. & Dolan, V. F. Albumin and all-cause mortality risk in insurance applicants. *J. Insur. Med.* **42**, 11–17 (2010).
41. Barbieri, M. et al. Higher circulating levels of IGF-1 are associated with longer leukocyte telomere length in healthy subjects. *Mech. Ageing Dev.* **130**, 771–776 (2009).
42. Junnila, R. K., List, E. O., Berryman, D. E., Murrey, J. W. & Kopchick, J. J. The GH/IGF-1 axis in ageing and longevity. *Nat. Rev. Endocrinol.* **9**, 366–376 (2013).
43. Putman, A. K., Brown, J. L., Gandy, J. C., Wisniewski, L. & Sordillo, L. M. Changes in biomarkers of nutrient metabolism, inflammation, and oxidative stress in dairy cows during the transition into the early dry period. *J. Dairy Sci.* **101**, 9350–9359 (2018).
44. van der Spek, A. et al. Fat metabolism is associated with telomere length in six population-based studies. *Hum. Mol. Genet.* **31**, 1159–1170 (2022).

45. Gavia-García, G. et al. Telomere length and oxidative stress and its relation with metabolic syndrome components in the aging. *Biology (Basel)* **10**, 253 (2021).
46. Contreras, G. A. & Sordillo, L. M. Lipid mobilization and inflammatory responses during the transition period of dairy cows. *Comp. Immunol. Microbiol. Infect. Dis.* **34**, 281–289 (2011).
47. Percie du Sert, N. et al. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol.* **18**, e3000411 (2020).
48. Van Duinkerken, G. et al. Update of the Dutch protein evaluation system for ruminants: The DVE/OEB 2010 system. *J. Agric. Sci.* **149**, 351–367 (2011).
49. Van Es, A. J. H. Feed evaluation for dairy cows. *Livest. Prod. Sci.* **2**, 95–107 (1975).
50. Mullins, I. L., Truman, C. M., Campler, M. R., Bewley, J. M. & Costa, J. H. C. Validation of a commercial automated body condition scoring system on a commercial dairy farm. *Animals* **9**, 287 (2019).
51. Martens, D. S., Plusquin, M., Gyselaers, W., De Vivo, I. & Nawrot, T. S. Maternal pre-pregnancy body mass index and newborn telomere length. *BMC Med.* **14**, 148 (2016).
52. Meesters, M. et al. Prenatal environment impacts telomere length in newborn dairy heifers. *Sci. Rep.* **13**, 4672 (2023).
53. Cawthon, R. M. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res.* **37**, e21–e21 (2009).
54. Cawthon, R. M., Smith, K. R., O'Brien, E., Sivatchenko, A. & Kerber, R. A. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* **361**, 393–395 (2003).
55. Hellemans, J., Mortier, G., De Paepe, A., Speleman, F. & Vandesompele, J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol.* **8**, R19 (2007).
56. Hernández, F., Madrid, J., García, V., Orengo, J. & Megias, M. D. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. *Poult. Sci.* **83**, 169–174 (2004).
57. Grotto, D. et al. Rapid quantification of malondialdehyde in plasma by high performance liquid chromatography–visible detection. *J. Pharm. Biomed. Anal.* **43**, 619–624 (2007).
58. Nimalaratne, C., Lopes-Lutz, D., Schieber, A. & Wu, J. Free aromatic amino acids in egg yolk show antioxidant properties. *Food Chem.* **129**, 155–161 (2011).
59. Degroote, J., Michiels, J., Claeys, E., Olyn, A. & De Smet, S. Changes in the pig small intestinal mucosal glutathione kinetics after weaning. *J. Anim. Sci.* **90**, 359–361 (2012).
60. Johnson, R. N., Metcalf, P. A. & Baker, J. R. Fructosamine: A new approach to the estimation of serum glycosylprotein. An index of diabetic control. *Clinica Chimica Acta* **127**, 87–95 (1983).
61. Pollack, A. Z., Rivers, K. & Ahrens, K. A. Parity associated with telomere length among US reproductive age women. *Hum. Reprod.* **33**, 736–744 (2018).

Acknowledgements

The authors acknowledge the University of Ghent and University of Hasselt for providing computational resources and support that have contributed to obtaining the research results reported in the present publication. The authors gratefully acknowledge the Research Foundation—Flanders (FWO) for the funding.

Author contributions

Conceptualization: Manon Dewulf, Geert Opsomer; Methodology: Manon Dewulf, Dries Martens, Stijn Heirbaut; Data curation: Manon Dewulf, Stijn Heirbaut, Mingqi Zhang, Maya Meesters and Mieke Van Eetvelde; Formal analysis: Manon Dewulf, Osvaldo Bogado Pascottini, Stijn Heirbaut, Dries Martens; Writing—original draft: Manon Dewulf; Writing review & editing: Geert Opsomer, Osvaldo Bogado Pascottini, Stijn Heirbaut, Maya Meesters, Tim Nawrot, Dries Martens, Leen Vandaele, Veerle Fievez; General supervision: Geert Opsomer.

Funding

The present study was funded by a PhD grant from the Research Foundation—Flanders (FWO grant 1SH5N24N). Dries S. Martens holds a postdoctoral grant by the Research Foundations Flanders (FWO grant 12X9623N). We acknowledge the financial support of Flanders Innovation & Entrepreneurship (VLAIO) (VLAIO; Belgium; LA170830). The research of Xiaoping Jing and Mingqi Zhang was supported by the Chinese Scholarship Council (CSC, China); the PhD research of Stijn Heirbaut was funded by a PhD grant from the Special Research Fund of the Ghent University (Bijzonder Onderzoeksfonds, BOF, Belgium). This research did not receive any specific grant from other funding agencies in the public, commercial, or not-for-profit sectors.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-024-82664-5>.

Correspondence and requests for materials should be addressed to M.D.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2024