# Relationship between inflammatory biomarkers and oxidative stress with uterine health in dairy cows with different dry period lengths[1]

**Novi Mayasari,[†,‡,2] Erminio Trevisi,[‖] Annarita Ferrari,[‖] Bas Kemp,[†] Henk K. Parmentier,[†] and Ariette T.M. van Knegsel[†]**

[†]Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, Wageningen, the Netherlands; [‡]Faculty of Animal Husbandry, Universitas Padjadjaran, Bandung, Indonesia; and [‖]Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, Piacenza, Italy

**ABSTRACT:** Earlier studies indicated that the inflammatory status of dairy cows in early lactation could not be fully explained by the negative energy balance (NEB) at that moment. The objective of the present study was to determine relationships between inflammatory biomarkers and oxidative stress with uterine health in dairy cows after different dry period lengths. Holstein–Friesian dairy cows were assigned to one of three dry period lengths (0-, 30-, or 60-d) and one of two early lactation rations (glucogenic or lipogenic ration). Cows were fed either a glucogenic or lipogenic ration from 10-d before the expected calving date. Part of the cows which were planned for a 0-d dry period dried themselves off and were attributed to a new group (0 → 30-d dry period), which resulted in total in four dry period groups. Blood was collected ($N$ = 110 cows) in weeks −3, −2, −1, 1, 2, and 4 relative to calving to determine biomarkers for inflammation, liver function, and oxidative stress. Uterine health status (UHS) was monitored by scoring vaginal discharge (VD) based on a 4-point scoring system (0, 1, 2, or 3) in weeks 2 and 3 after calving. Cows were classified as having a healthy uterine environment (HU, VD score = 0 or 1 in both weeks 2 and 3), nonrecovering uterine environment (NRU, VD score = 2 or 3 in week 3), or a recovering uterine environment (RU, VD score = 2 or 3 in week 2 and VD score= 0 or 1 in week 3). Independent of dry period length, cows with NRU had higher plasma haptoglobin ($P$ = 0.05) and lower paraoxonase levels ($P$ < 0.01) in the first 4 weeks after calving and lower liver functionality index ($P$ < 0.01) compared with cows with HU. Cows with NRU had lower plasma albumin ($P$ = 0.02) and creatinine ($P$ = 0.02) compared with cows with a RU, but not compared with cows with HU. Independent of UHS, cows with a 0 → 30-d dry period had higher bilirubin levels compared with cows with 0-, 30-, or 60-d dry period ($P$ < 0.01). Cows with RU and fed a lipogenic ration had higher levels of albumin in plasma compared with cows with NRU and fed a lipogenic ration ($P$ < 0.01). In conclusion, uterine health was related to biomarkers for inflammation (haptoglobin and albumin) and paraoxonase in dairy cows in early lactation. Cows which were planned for a 0-d dry period, but dried themselves off (0 → 30-d dry period group) had higher bilirubin levels, which was possibly related to a more severe NEB in these cows. Inflammatory biomarkers in dairy cows in early lactation were related to uterine health in this period.

**Key words:** cattle, continuous milking, inflammation, oxidative stress, uterine health

[1]This work is part of the PhD thesis of Novi Mayasari which she defended at Wageningen University. We thank the Directorate General of Higher Education, Ministry of National Education, Jakarta, Indonesia for the scholarship of Novi Mayasari. We thank the Dutch Dairy Board (PZ, Zoetermeer, the Netherlands), the Product Board Animal Feed (PDV, Zoetermeer, the Netherlands), and CRV (Arnhem, the Netherlands), for financing the experiment.

[2]Corresponding author: novi.mayasari@unpad.ac.id

Received February 5, 2019.

Accepted May 8, 2019.

*© The Author(s) 2019. Published by Oxford University Press on behalf of the American Society of Animal Science.*
*This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com*

Transl. Anim. Sci. 2019.3:607–619
doi: 10.1093/tas/txz040

## INTRODUCTION

Shortening and omitting the dry period is of interest because it has the potential to improve the energy balance (EB) (Rastani et al., 2005; de Feu et al., 2009; Van Knegsel et al., 2014), metabolic status (Rastani et al., 2005; Chen et al., 2015a), and fertility (de Feu et al., 2009; Chen et al., 2015b) of dairy cows in the next lactation. The improvement of the EB is mainly due to a reduction in milk yield in the subsequent lactation (Van Knegsel et al., 2014), and sometimes also to an improvement of the dry matter intake (DMI) in the peripartum period (Rastani et al., 2005). Shortening or omitting of the dry period, however, for a second lactation resulted in a reduction in milk yield losses, disappearance of the difference in EB, and a different effect on metabolic status, compared with the first lactation after implementation of dry period length treatments. Difference in cow metabolism and productive performance between first and second lactation after implementation of dry period length treatments was discussed to be related to aging of the cows, increase in body condition, and spontaneous drying off of cows which were planned for a second 0-d dry treatment (Chen et al., 2016).

Difference in EB and metabolic status between first and second lactation after implementation of dry period length treatments can be expected to affect inflammatory status of dairy cows in early lactation. In the first lactation after implementation of dry period length treatments, a 0-d dry period resulted in increased levels of ceruloplasmin, cholesterol, reactive oxygen metabolites (ROM) and decreased levels of bilirubin and paraoxonase in plasma, and decreased liver functionality index (LFI) compared with cows with a 60-d dry period (Mayasari et al., 2017). The effects of dry period length on inflammatory status could partly be explained by the improved EB (lower bilirubin) and by the occurrence of health problems (greater ceruloplasmin and lower ROM) in cows with a 0-d dry period (Mayasari et al., 2017), compared with cows with a 30- or 60-d dry period. In this earlier study, investigated clinical health problems included clinical mastitis, fever, metritis, and retained placenta (Mayasari et al., 2017). It can be hypothesized that not only clinical health problems, but also subclinical inflammatory processes, like recovery of the uterus postpartum, could clarify alterations in inflammatory and oxidative stress variables in dairy cows in early lactation after a 0-d dry period.

In early lactation, a severe negative energy balance (NEB) has been related to a greater risk for uterine health problems (Hammon et al., 2006; Manimaran et al., 2016). During infection of the uterus, proinflammatory cytokines alter the acute phase protein (APP) concentrations in plasma (Baumann and Gauldie, 1994; Trevisi et al., 2008; Moretti et al., 2015) and negatively affect uterine immunity (Manimaran et al., 2016). Uterus palpation and monitoring of vaginal discharge (VD) have been widely used to diagnose uterine health problems in dairy cows (LeBlanc et al., 2002; Gilbert et al., 2005; Williams et al., 2005; Prunner et al., 2014). VD has been related to high plasma haptoglobin levels (Huzzey et al., 2009; Dubuc et al., 2010; Ametaj et al., 2014). The relationship between uterine health status (UHS), as indicated by VD, and oxidative stress is unclear. Previous studies showed that VD was associated with increased oxidative stress (Kizil et al., 2010; Magata et al., 2017), but not all (Bicalho et al., 2014). We hypothesize that an altered inflammatory status in early lactation of cows with a 0-d dry period, compared with cows with a 30- or a 60-d dry period, is possibly not only related to improved EB and occurrence of clinical health problems (Mayasari et al., 2017), but also related to recovery of the uterus in this phase of lactation, indicated by VD.

In earlier studies, we found that a glucogenic ration, compared with a lipogenic ration, improved metabolic status, as indicated by lower free fatty acid and β-hydroxybutyrate levels in plasma (Chen et al., 2015a) and shortened the interval from calving to onset of luteal activity (Chen et al., 2015b, 2017) of cows with different dry period lengths. It can be hypothesized that the beneficial effects of a glucogenic ration on EB and metabolic status are also related to a reduction in inflammatory response after a shortened or omitted dry period.

To the best of our knowledge, the relationships between inflammatory biomarkers and oxidative

stress with uterine health in dairy cows with different dry period lengths are unknown. The objective of this study was to determine the relationships between inflammatory biomarkers and oxidative stress with uterine health in dairy cows in early lactation after implementation of different dry period lengths.

## MATERIALS AND METHODS

### *Housing, Animals, Experimental Design, and Rations*

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol of this study. The registration number of the experimental protocol is 2010026. Cows were housed in a freestall with slatted floor and cubicles and milked twice daily (at 0500 and 1630 h). The experimental design, treatments of dry period lengths, and ration composition were described earlier by Van Knegsel et al. (2014). In short, Holstein–Friesian dairy cows ($N = 167$, 60 primiparous and 107 multiparous) were selected from the Dairy Campus Research herd (WUR Livestock Research, Lelystad, the Netherlands), blocked according to parity, expected calving date, milk yield in the previous lactation, and body condition score (BCS), and randomly assigned to treatments within blocks. Treatments consisted of three dry period lengths (0-, 30-, or 60-d) and two early lactation rations (glucogenic or lipogenic) resulting in a 3 × 2 factorial design. Cows were planned to have the same dry period length and dietary energy source over two subsequent lactations. After the first lactation of implementation of dry period length treatments, 17 cows with a 0-d dry period, 14 cows with a 30-d dry period, and 6 cows with a 60-d dry period were excluded due to health problems or not being pregnant. Therefore, the second lactation after implementation of dry period length treatments started with 130 cows: 39 cows with a 0-d dry period, 41 cows with a 30-d dry period, and 50 cows with a 60-d dry period. Moreover, 19 cows in the 0-d dry period group were attributed before the second lactation after implementation of dry period length treatments to a new group because these cows had very low milk yield of <4 kg/d at least 30 d before expected calving date (0 → 30-d dry period; actual day dry: 67 ± 8 d). In the second lactation after implementation of dry period length treatments, blood was collected weekly from 110 cows. In the second lactation after implementation of dry period length treatments, VD was collected and scored in weeks 2 and 3 after calving from 91 cows according to Williams et al. (2005). The drying-off protocol was described in an earlier study (Van Knegsel et al., 2014). In short, the drying off protocol for cows with the 30- and 60-d dry period consisted of a transition to the far-off ration at day 7 before drying-off, and milking once daily at day 4 before drying-off cows and were treated with an intramammary antibiotic at drying off (Supermastidol Virbac Animal Health, Barneveld, the Netherlands). Cows in the 0 → 30-d dry period group were dried off by transferring to the dry cow ration and without use of intramammary antibiotics.

Ration composition and feeding strategies were described earlier (Van Knegsel et al., 2014). In short, cows with a 30- or a 60-d dry period received prepartum a dry cow ration, whereas cows with a 0-d dry period received prepartum a lactating cow ration supporting 25 kg of milk yield per day. From 10-d before the expected calving date onwards, cows of all treatments were fed 1 kg/d of the glucogenic or lipogenic concentrate, which was increased postcalving in steps of 0.5 kg/d until the concentration supply reached 8.5 kg/d. The main ingredient for the glucogenic concentrate was corn and the main ingredients for the lipogenic concentrate were sugar beet pulp, palm kernel, and rumen-protected palm oil. Experimental concentrate was provided individually over six periods within 24 h by a computerized feeder located in the freestall that was available to all cows at all times (Manus VC5, DeLaval, Steenwijk, the Netherlands). Concentrate and forage were supplied separately. Forage composition that did not differ among rations was supplied ad libitum and described earlier in detail (Van Knegsel et al., 2014). In short, before calving, forage consisted of grass silage, corn silage, wheat straw, and rapeseed meal or soybean meal (39:25:25:11 on DM basis). After calving, forage consisted of grass silage, corn silage, wheat straw, and rapeseed meal or soybean meal (51:34:2:13 on DM basis). Rations were isocaloric (net energy basis: Dutch net energy evaluation [VEM] system; Van Es, 1975; CVB, 2005) and contained equal amounts of intestinal digestible protein and rumen degradable protein (DVE/OEB; Tamminga et al., 1994).

### *Blood Sampling*

Blood sampling was described earlier (Chen et al., 2015a). In short, blood samples were taken weekly from the tail vein at 3 h before the morning feeding from week −3 to 4 relative to calving. Blood was collected in evacuated tubes (Vacuette, Greiner BioOne, Kremsmunster, Austria) containing lithium-heparin for haptoglobin, ceruloplasmin, cholesterol, albumin, bilirubin, paraoxonase, ROM, ferric reducing antioxidant power (FRAP),

and creatinine. Samples were kept cold on ice for a maximum of 2 h until they were centrifuged at 3,000 × *g* for 15 min at 4°C. Plasma was decanted, aliquoted, and frozen at −20°C until analysis.

### *Laboratory Analysis*

Determination of inflammatory biomarkers and oxidative stress variables was described earlier (Mayasari et al., 2017). In short, inflammatory biomarkers and oxidative stress were measured at Faculty of Agriculture, Food and Environmental Sciences, Università Cattolica del Sacro Cuore in Piacenza, Italy, following the procedures previously described by Bionaz et al. (2007) and evaluated by Calamari et al. (2016) and Jacometo et al. (2015) using a clinical auto-analyzer (ILAB 650, Instrumentation Laboratory, Lexington, MA). Total cholesterol (catalog no. 0018250540), albumin (catalog no. 0018250040), total bilirubin (catalog no. 0018254640), and creatinine (catalog no. 0018255540) were measured using the IL Test purchased from Instrumentation Laboratory Spa (Werfen Co., Milan, Italy). Haptoglobin was determined with the method described by Skinner et al. (1991) and Owen et al. (1960), adapted to ILAB 650 condition. Haptoglobin determination was based on peroxidase activity of methaemoglobin–haptoglobin complex measured by the rate of oxidation of guaiacol (hydrogen donor) in the presence of hydrogen peroxide (oxidizing substrate). Ceruloplasmin was determined with the method described by Sunderman and Nomoto (1970), adapted to ILAB 650 conditions. The test is based on measurement of the color which originates from the oxidation of the p-phenylenediamine dihydrochloride induced by the ceruloplasmin. Reactive oxygen metabolite was measured using commercial kits (Diacron International s.r.l., Grosseto, Italy, kit d-ROMs-test cod. MC003) adapted to the ILAB 650 conditions. Antioxidant potential was assessed as FRAP using the colorimetric method of Benzie and Strain (1996). Plasma paraoxonase activity was measured by adapting the method of Ferré et al. (2002) to the ILAB 650 conditions. In this study, CV intraassay for haptoglobin, ceruloplasmin, total bilirubin, total cholesterol, albumin, paraoxonase, FRAP, and ROM was 5.9, 0.7, 1.4, 0.7, 0.3, 2.2, 3.4, and 1.6, respectively. In addition, CV interassay for haptoglobin, ceruloplasmin, total bilirubin, total cholesterol, albumin, paraoxonase, FRAP, and ROM was 5.2, 4.2, 6.7, 3.9, 2.5, 6.5, 5.3, and 4.5, respectively.

### *Vaginal Discharge Collection and Scoring*

VD collection was described earlier (Chen et al., 2017) and scored in weeks 2 and 3 after calving from 91 cows according to Williams et al. (2005). In short, VD was scored based on a 4-point scoring system ranging from score 0 (clear discharge) to score 3 (discharge containing > 50% purulent material). UHS was defined based on the VD score in weeks 2 and 3 after calving: a healthy uterine environment (HU, VD score = 0 or 1 in both weeks 2 and 3), nonrecovering uterine environment (NRU, VD score = 2 or 3 in week 3), or a recovering uterine environment (RU, VD score = 2 or 3 in week 2 and VD score = 0 or 1 in week 3).

### *Statistical Analyses*

The MIXED procedure of SAS (SAS version 9.2; SAS Institute Inc., Cary, NC; Littell et al., 1996) for repeated measures analysis was used to analyze the effects of dry period lengths and rations on inflammatory biomarkers and oxidative stress (model 1). Dependent variables in model 1 were haptoglobin, ceruloplasmin, albumin, cholesterol, paraoxonase, bilirubin, ROM, FRAP, creatinine, and LFI. Fixed effects in model 1 were dry period length (0, 30, 60, or 0 → 30 d), ration (glucogenic or lipogenic), parity (3, 4, or >4), week relative to calving (−3, −2, −1, 1, 2, and 4), and their 2-way interactions. Albumin, cholesterol, and bilirubin data were used to calculate the LFI. Statistical analysis for LFI was performed in the first 4 weeks after calving with model 1.

The MIXED procedure of SAS was used to analyze the relation of UHS with inflammatory biomarkers and oxidative stress (model 2). Dependent variables in model 2 were haptoglobin, ceruloplasmin, albumin, cholesterol, paraoxonase, bilirubin, ROM, FRAP, creatinine, and LFI. Fixed effects in model 2 were UHS (HU, NRU, or RU), dry period length (0, 30, 60, or 0 → 30 d), ration (glucogenic or lipogenic), parity (3, 4, or >4), week relative to calving (−3, −2, −1, 1, 2, and 4), and their 2-way interactions in separate analyses one by one.

In both models 1 and 2, a repeated effect was included with cow as the repeated subject. A first-order autoregressive covariance matrix (AR (1)) was the best fit according to Akaike's corrected information criterion and was used to account for within-cow variation. Preliminary analysis showed that number of cows per category of UHS × dry period length was small (ranging from 1 to 15); thus, this interaction was not included in model

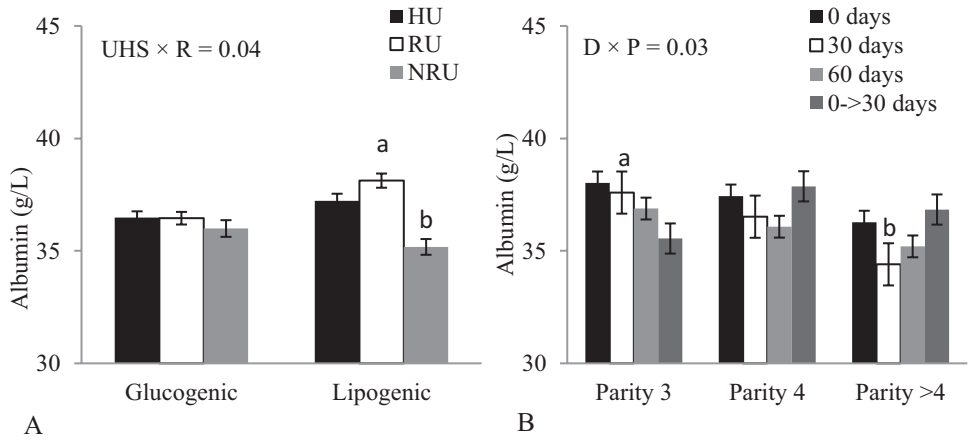


**Figure 1.** (A) Albumin concentration in plasma for cows with different UHS (HU, RU, or NRU) and fed either a glucogenic or lipogenic ration. (B) Albumin concentration in plasma of cows with different dry period lengths (0-, 30-, 0 → 30-, or 60-d dry period) and different parities (3, 4, or >4). Values represent means ± SEM. Bar with different superscripts within dry period length (D), ration (R), or parity (P) class differ ($P < 0.05$).

2. For comparison of dry period length effects, $P$ values are presented after a Tukey–Kramer adjustment. Values are presented as least squares means (LSM) with their pooled standard errors of the mean (SEM), unless otherwise stated. Differences are regarded as significant if $P < 0.05$, and trends are discussed if $P \leq 0.10$.

## RESULTS

### *Effects of Dry Period Length and Ration on Inflammatory Biomarkers, Liver Functionality, and Oxidative Stress in Plasma of Cows*

Cows with a 0-d dry period had higher levels of ceruloplasmin in plasma ($P < 0.04$, Table 1) and higher LFI ($P < 0.01$, Table 1) compared with cows with a 0 → 30-, 30-, or 60-d dry period from 3 weeks before calving until 4 weeks after calving. Cows with a 0-d dry period had higher levels of ROM in plasma compared with cows with a 30- ($P = 0.03$) or 60-d dry period ($P = 0.01$) from 3 weeks before calving until 4 weeks after calving, but not compared with cows with a 0 → 30-d dry period ($P = 0.28$). Cows with a 0 → 30-d dry period had higher bilirubin levels in plasma compared with cows with a 0-, 30-, or 60-d dry period especially in the first 2 weeks after calving ($P < 0.01$, Table 1). Plasma bilirubin levels did not differ among cows with a 0-, 30-, or 60-d dry period ($P = 0.99$, $P = 0.98$, and $P = 1.00$, respectively). Cows with a 0 → 30-d dry period had higher creatinine levels in plasma compared with cows with a 60-d dry period ($P < 0.01$), but not compared with cows a 0-d ($P = 0.61$) and 30-d dry period ($P = 0.10$). Effect of dry period length on albumin was depended on parity ($P = 0.04$, Table 1, Figure 1). Cows with 0 → 30-d dry period had higher albumin levels in plasma in cows with parity 4 compared with cows with parity 3 ($P = 0.04$). Contrary, cows with a 0-, a 30-, or a 60-d dry period had lower albumin levels in plasma in cows with parity ≥ 4 compared with cows with parity 3 ($P < 0.01$). Dry period length had no effect on plasma levels of haptoglobin, cholesterol, paraoxonase, or FRAP ($P = 0.65$, $P = 0.13$, and $P = 0.29$, respectively, Table 1).

Cows fed a lipogenic ration had higher cholesterol levels in plasma ($P = 0.04$, Table 1) and higher LFI ($P < 0.01$, Table 1) compared with cows fed a glucogenic ration. There was an interaction between dry period × ration for FRAP and creatinine levels ($P = 0.03$ and $P < 0.01$, respectively, Table 1). Cows with a 60-d dry period fed a lipogenic ration had higher FRAP ($P = 0.02$) and creatinine levels ($P = 0.04$) in plasma compared with cows with a 60-d dry period and fed a glucogenic ration. Ration had no effect on levels of haptoglobin, ceruloplasmin, bilirubin, paraoxonase, ROM, or creatinine in plasma ($P = 0.45$, 0.45, 0.22, 0.36, 0.20, and 0.61, respectively, Table 1).

### *Relationships of Uterine Health Status With Inflammatory Biomarkers and Oxidative Stress*

Cows with NRU had higher haptoglobin ($P = 0.05$) levels in plasma compared with cows with HU, but not compared with cows with RU ($P = 0.86$), independent of dry period length ($P = 0.01$, Table 2). Cows with NRU had lower paraoxonase ($P < 0.01$, Table 2) levels in plasma and lower LFI ($P < 0.01$, Table 2) compared with cows with HU or RU, independent of dry period length. In addition, cows with NRU had lower albumin ($P = 0.02$) and creatinine ($P < 0.01$) levels in plasma compared with cows with a RU, but

**Table 1.** Inflammatory biomarkers (haptoglobin, ceruloplasmin, albumin, and cholesterol), index of liver function (paraoxonase and bilirubin), oxidative stress (ROM and FRAP), creatinine, and liver functionality index in plasma of dairy cows with different dry period lengths (0, 30, 60, or 0 → 30* d) and fed either a glucogenic (G) or a lipogenic (L) ration (LSM ± pooled SEM)

| | Dry period length, d | | | | | Ration | | | $P^{\dagger}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 30 | 60 | 0 → 30* | SEM | G | L | SEM | D | R | P | W‡ | D × R | D × P | D × W | P × R |
| Cows, $n$ = 110 | 15 | 32 | 44 | 19 | | 56 | 54 | | | | | | | | | |
| Haptoglobin, g/L | 0.47 | 0.45 | 0.46 | 0.51 | 0.04 | 0.49 | 0.45 | 0.03 | 0.65 | 0.45 | 0.76 | <0.01 | 0.58 | 0.65 | 0.12 | 0.94 |
| Ceruloplasmin, µmol/L | 2.01[a] | 1.75[b] | 1.80[b] | 1.81[b] | 0.09 | 1.89 | 1.79 | 0.06 | 0.04 | 0.45 | 0.15 | <0.01 | 0.42 | 0.96 | 0.11 | 0.30 |
| Albumin, g/L | 37.37 | 36.12 | 36.09 | 36.86 | 0.53 | 36.21 | 37.01 | 0.37 | 0.20 | 0.21 | 0.08 | <0.01 | 0.34 | 0.04 | 0.34 | 0.19 |
| Cholesterol, mmol/L | 3.02 | 2.93 | 2.65 | 2.65 | 0.11 | 2.75 | 2.88 | 0.07 | 0.13 | 0.04 | <0.01 | <0.01 | 0.71 | 0.65 | 0.34 | 0.26 |
| Bilirubin, µmol/L | 0.94[a] | 1.10[a] | 1.14[a] | 1.92[b] | 0.15 | 1.22 | 1.33 | 0.08 | <0.01 | 0.22 | 0.74 | <0.01 | 0.52 | 0.99 | <0.01 | 0.21 |
| Paraoxonase, U/mL | 46.54 | 48.05 | 47.00 | 51.68 | 2.76 | 47.48 | 49.16 | 1.94 | 0.50 | 0.36 | 0.08 | <0.01 | 0.08 | 0.75 | 0.01 | 0.64 |
| ROM, mg $H_2O_2$/100 mL | 16.17[a] | 14.84[b] | 14.79[b] | 15.24[ab] | 0.39 | 15.52 | 15.01 | 0.27 | 0.01 | 0.20 | 0.07 | <0.01 | 0.74 | 0.46 | 0.04 | 0.24 |
| FRAP, µmol/L | 156.44 | 147.76 | 151.51 | 148.34 | 3.15 | 152.90 | 149.12 | 2.39 | 0.29 | 0.06 | <0.01 | <0.01 | 0.03 | 0.14 | 0.62 | 0.07 |
| Creatinine, µmol/L | 106.21[ab] | 103.92[ab] | 98.54[a] | 109.83[b] | 2.13 | 104.02 | 105.23 | 1.39 | <0.01 | 0.61 | 0.04 | 0.04 | <0.01 | 0.55 | 0.32 | 0.44 |
| Liver functionality index | 1.41[a] | 0.38[b] | 0.55[b] | −0.05[b] | 0.21 | −0.22 | 1.37 | 0.15 | <0.01 | <0.01 | <0.01 | NA | <0.01 | <0.01 | NA | 0.02 |

[a,b]Values within dry period length in the same row with different superscripts differ ($P < 0.05$).

*In the second subsequent lactation, 19 cows, which were planned to have a 0-d dry period, had a milk production of <4 kg/d for at least 30 d before the expected calving date and were allowed to go dry.

†D = dry period; R = ration; P = parity; NA = not applicable.

‡Weeks −3, −2, −1, 1, 2, and 4 relative to calving.

**Table 2.** Inflammatory biomarkers (haptoglobin, ceruloplasmin, albumin, and cholesterol), index of liver function (paraoxonase and bilirubin), oxidative stress (ROM and FRAP), creatinine, and LFI in plasma of dairy cows with varying dry period lengths (0, 30, 60, or 0 → 30* d) and fed either a glucogenic (G) or a lipogenic (L) ration categorized according to UHS (LSM ± pooled SEM)

| | UHS[†] | | | | Dry period length, d | | | | | *P*[‡] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | HU | RU | NRU | SEM | 0 | 30 | 60 | 0 → 30* | SEM | UHS | D | R | P | W | D × P | D × W | P × R | UHS × R |
| Cows, *n* = 91 | 17 | 31 | 43 | | 12 | 29 | 36 | 14 | | | | | | | | | | |
| Haptoglobin, g/L | 0.41[b] | 0.44[ab] | 0.56[a] | 0.04 | 0.48 | 0.46 | 0.47 | 0.46 | 0.05 | 0.01 | 0.99 | 0.78 | 0.84 | <0.01 | 0.87 | 0.35 | 0.93 | 0.25 |
| Ceruloplasmin, µmol/L | 2.00 | 1.86 | 1.97 | 0.09 | 2.09 | 1.83 | 1.95 | 1.91 | 0.10 | 0.39 | 0.38 | 0.85 | 0.28 | <0.01 | 0.11 | 0.03 | 0.43 | 1.00 |
| Albumin, g/L | 37.22[ab] | 37.28[b] | 35.62[a] | 0.54 | 36.44 | 36.70 | 36.39 | 37.30 | 0.62 | 0.01 | 0.65 | 0.88 | 0.26 | <0.01 | 0.03 | 0.48 | 0.02 | 0.04 |
| Cholesterol, mmol/L | 2.90 | 2.97 | 2.78 | 0.15 | 3.25 | 2.86 | 2.75 | 2.68 | 0.17 | 0.50 | 0.22 | 0.37 | <0.01 | <0.01 | 0.82 | 0.73 | 0.89 | 0.71 |
| Bilirubin, µmol/L | 1.22 | 1.25 | 1.27 | 0.15 | 0.73[a] | 1.22[a] | 1.17[a] | 1.86[b] | 0.18 | 0.98 | <0.01 | 0.39 | 0.35 | <0.01 | 0.69 | <0.01 | 0.08 | 0.40 |
| Paraoxonase, U/mL | 59.83[a] | 52.40[a] | 43.30[b] | 2.73 | 50.38 | 51.92 | 48.24 | 56.83 | 3.14 | <0.01 | 0.15 | 0.06 | 0.12 | <0.01 | 0.10 | 0.49 | 0.73 | 0.07 |
| ROM, mg $H_2O_2$/100mL | 15.69 | 15.22 | 15.85 | 0.36 | 16.70 | 15.13 | 15.27 | 15.25 | 0.41 | 0.28 | 0.13 | 0.53 | 0.03 | <0.01 | 0.18 | 0.08 | 0.57 | 0.11 |
| FRAP, µmol/L | 157.35 | 148.03 | 145.87 | 3.49 | 152.55 | 147.75 | 152.86 | 148.51 | 4.01 | 0.09 | 0.57 | 0.34 | <0.01 | <0.01 | 0.18 | 0.58 | 0.41 | 0.64 |
| Creatinine, µmol/L | 104.40[ab] | 106.84[b] | 100.45[a] | 1.95 | 101.49[ab] | 105.57[a] | 98.80[b] | 109.73[a] | 2.25 | 0.02 | <0.01 | 0.53 | 0.52 | <0.01 | 0.46 | 0.31 | 0.02 | 0.60 |
| LFI | 1.19[a] | 1.32[a] | −0.34[b] | 0.22 | 1.91[a] | 0.36[b] | 0.58[b] | 0.04[b] | 0.26 | <0.01 | <0.01 | <0.01 | <0.01 | NA | <0.01 | NA | <0.01 | 0.26 |

[a,b]Values within dry period length in the same row with different superscripts differ ($P < 0.05$).

*In the second subsequent lactation, 19 cows, which were planned to have a 0-d dry period, had a milk production of <4 kg/d for at least 30 d before the expected calving date and were allowed to go dry.

[†]HU = healthy uterine environment: vaginal discharge (VD) score = 0 or 1 in both week 2 and 3; RU = a recovering uterine environment: VD score = 2 or 3 in week 2 and score = 0 or 1 in week 3; NRU = Non-recovering uterine environment: VD score: 2 or 3 in week 3.

[‡]D = dry period; R = ration; P = parity; NA = not applicable; D × R, UHS × W and UHS × P are included in the model, but not significant. UHS × D are excluded from the model because of fewer animals in the group.

not compared with cows with HU ($P < 0.01$). There were interactions between UHS × ration ($P = 0.04$, Table 2) and dry period length × parity ($P = 0.03$, Table 2) for plasma albumin levels. Cows with RU and fed a lipogenic ration had higher levels of albumin in plasma compared with cows with NRU and fed a lipogenic ration ($P < 0.01$). Cows with a 30-d dry period had higher levels of albumin in plasma in cows with parity 3 compared with cows with parity > 4 ($P = 0.04$). There was an interaction between UHS × week for haptoglobin ($P = 0.06$, Figure 2). In addition, cows with NRU had higher levels of haptoglobin ($P = 0.01$), lower albumin ($P = 0.01$), and paraoxonase ($P < 0.01$) in plasma compared with cows with HU or RU especially in the first 4 weeks after calving.

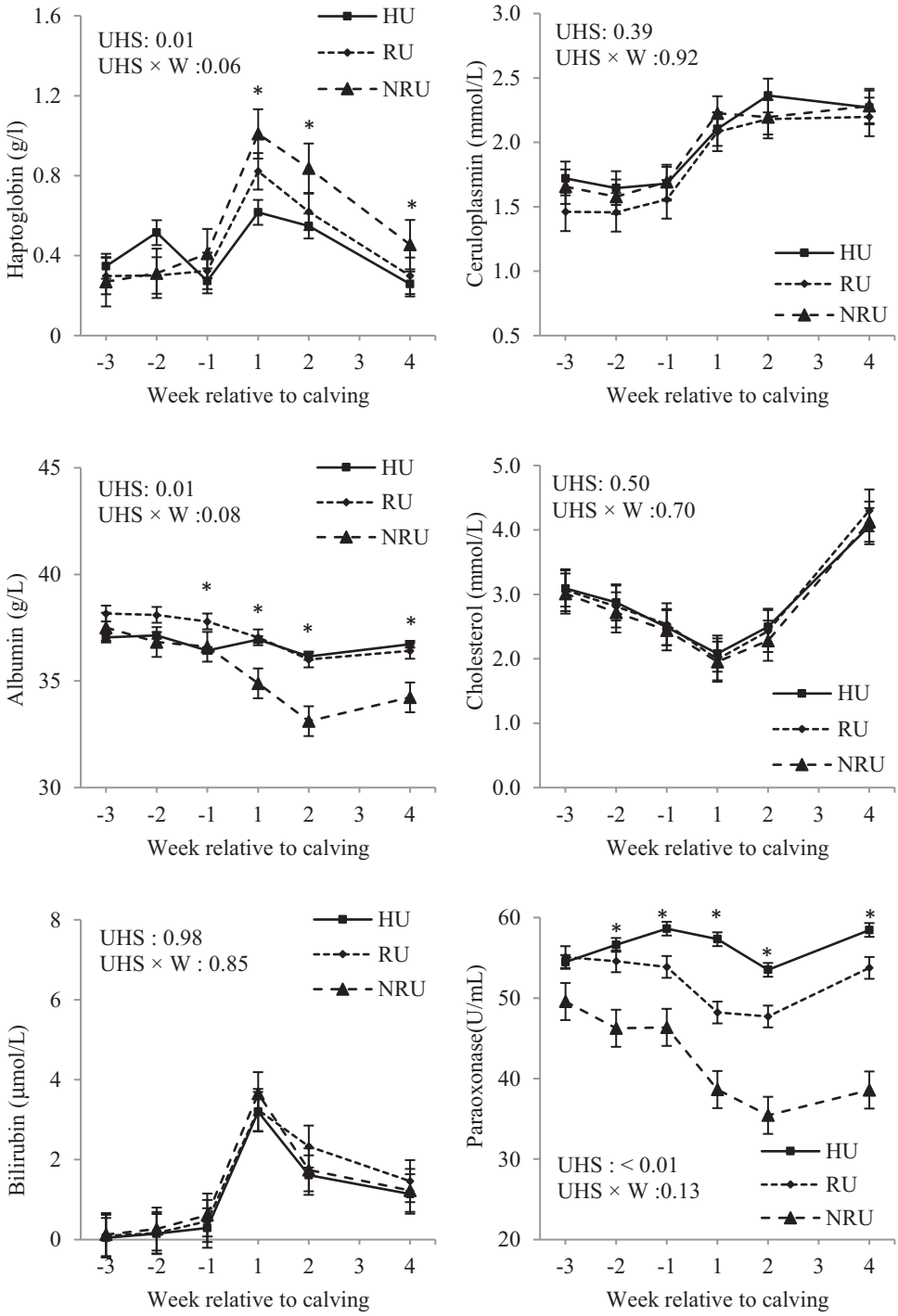


**Figure 2.** Inflammatory biomarkers, oxidative stress (ROM, and FRAP) and creatinine in plasma per week per class of UHS; HU; VD score: 0 or 1 in week 2 and score 0 or 1 in week 3 ($n = 17$), a RU; VD score: 2 or 3 in week 2 and score 0 or 1 in week 3 ($n = 31$), and a NRU; VD score: 2 or 3 in week 3 ($n = 43$) (Mean ± SEM). Significant UHS group differences within week are indicated by asterisk ($P < 0.05$).

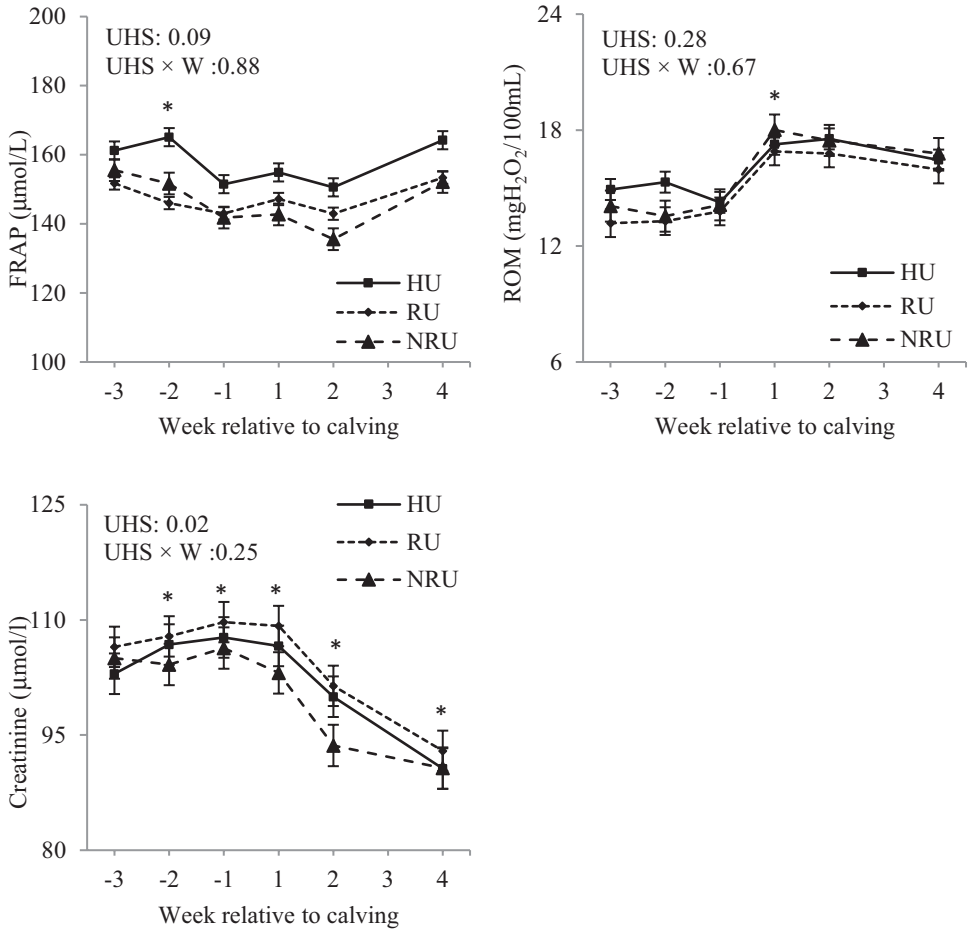


**Figure 2.** Continued

## DISCUSSION

In the current study, independent of dry period length or ration, cows with NRU had higher haptoglobin levels in plasma compared with cows with HU, but not compared with cows with RU. Cows with NRU had lower paraoxonase levels in plasma and LFI compared with cows with HU or RU, independent of dry period length. In addition, cows with NRU had lower albumin levels in plasma compared with cows with a RU, but not compared with cows with HU. Our finding is in line with a previous study where cows with an uterine infection had lower albumin, lower paraoxonase, and higher haptoglobin levels in plasma compared with cows with healthy uterus (Schneider et al., 2013). Cows with retained placenta and endometritis had lower levels of plasma albumin as negative APP (Fleck, 1989; Trevisi et al., 2008) compared with healthy cows (Green et al., 2009; Burke et al., 2010). Paraoxonase is mainly synthesized in the liver, considered as negative APP and a marker for liver functionality (Feingold et al., 1998). Low plasma paraoxonase level was associated with increased occurrence of clinical health disorders (e.g., retained placenta and metritis) in early lactation (Bionaz et al., 2007). Previous studies showed that cows with uterine infection had high haptoglobin levels in plasma (Chan et al., 2010; Hassanpour et al., 2011; Pohl et al., 2015). Plasma haptoglobin increased in cows with retained fetal membranes (Mordak, 2009). Retained fetal membranes is a key risk factor for uterine infection (Mordak, 2009). Total bilirubin, albumin, and vitamin A concentrations in plasma have been proposed as possible indicators for retained fetal membranes (Trevisi et al., 2008). Recently, the level of haptoglobin in plasma has been proposed as a biomarker to predict uterine infection in early lactation (Williams et al., 2005; Huzzey et al., 2009). Also in the current study, levels of haptoglobin in plasma were associated with UHS of cows after different dry period lengths.

In the present study, the increase of ceruloplasmin and ROM was affected by both dry period length and UHS. In our study, levels of ceruloplasmin were positively associated with levels of ROM in plasma ($r = 0.83$, $P < 0.01$). In addition, levels of ROM were negatively associated with level of

paraoxonase in plasma ($r = -0.19$, $P < 0.01$). It was previously observed that cows with acute puerperal metritis had increased oxidative stress, indicated by high malondialdehyde concentrations compared with healthy cows (Kizil et al., 2010; Magata et al., 2017). In earlier work, cows with endometritis had also higher ceruloplasmin levels in plasma, compared with healthy cows (Kaya et al., 2016). During inflammation, copper binding ceruloplasmin mediated formation of reactive oxygen species and may promote oxidative pathology (Shukla et al., 2006).

In the current study, cows with a 0-d dry period had higher ceruloplasmin and higher ROM levels in plasma compared with cows with a 60-d dry period, independent of UHS. In our earlier study, high levels of ceruloplasmin and ROM in plasma of cows with a 0-d dry period could partly be explained by the occurrence of clinical health problems related to inflammation such as clinical mastitis, metritis, retained placenta, and fever (Mayasari et al., 2017). In the current experiment, dry period length did not affect UHS (Chen et al., 2017). So albeit UHS is related to inflammation in the uterus (Ametaj et al., 2005; Bionaz et al., 2007; Trevisi et al., 2012) and might result in changes of inflammatory biomarkers and oxidative stress variables, UHS was not affected by dry period length treatment in the current study. This result indicated that high levels of ceruloplasmin and ROM in plasma of cows with 0-d dry period could not be explained by UHS but may be justified by other specific diseases related to inflammation in these cows.

Plasma bilirubin levels did not differ among cows with a 0- vs. 30- vs. 60-d dry period. Cows with 0 → 30-d dry period had higher plasma bilirubin levels compared with cows with 0-, 30-, or 60-d dry period. Cows with a 0 → 30-d dry period had a more severe NEB in early lactation compared with cows with a 0- or 30-d dry period (Chen et al., 2016). Moreover, cows with a 0-d dry period had a less pronounced improvement of EB and metabolic status in early lactation in the current study, compared with the first lactation after implementation of a short or no dry period strategy (Chen et al., 2016). In the first lactation after implementation of dry period length treatments, a better EB was related to low plasma bilirubin levels (Mayasari et al., 2017). The limited differences in EB among dry period groups in the second lactation after implementation of dry period length treatments may explain the lack of effect of dry period length on bilirubin, compared with the first lactation after implementation of dry period length treatments. In this study, the level of bilirubin was relatively low compared with earlier studies (Ametaj et al., 2005; Bionaz et al., 2007; Trevisi et al., 2012). The animal experiment of this study was performed from 2010 until 2013, and the prolonged storage condition of the plasma samples may have affected the level of bilirubin. In the current study, however, the dynamic of bilirubin levels by week around calving followed the typical pattern of change observed in the peripartum period in earlier studies (Ametaj et al., 2005; Bionaz et al., 2007; Trevisi et al., 2012).

In the present study, cows with NRU had lower creatinine levels compared with cows with RU and HU. This is in contrast with earlier studies where cows with uterine infection had higher creatinine in plasma compared with cows with a healthy uterus (Sattler and Fürll, 2004; Kaya et al., 2016). In our study, cows with a 0 → 30-d dry period had higher creatinine levels in plasma compared with cows with a 60-d dry period, independent of UHS. A previous study showed that cows with high BCS group (BCS ≥ 3.75) had higher plasma creatinine and more severe NEB compared with cows with low BCS group (BCS ≤ 2.5) (Pires et al., 2013). In the current experiment, cows with a 0 → 30-d dry period had higher BCS (4.3 ± 0.3) compared with cows with a 30-d dry period (3.4 ± 0.2) or 60-d dry period (3.2 ± 0.1) during 3 weeks before calving ($P = 0.02$). In addition, cows with a 0 → 30-d dry period had a more severe NEB compared with cows with a 0- or 30-d dry period (Chen et al., 2016). This result indicated that high plasma creatinine levels in cows with a 0 → 30-d dry period in this study was also related to high precalving BCS, suggesting that these cows also had a higher mass of muscle. This body "overcondition" before calving could be related to the overfeeding status in late pregnancy that previously has been related to a more severe postcalving NEB (Janovick et al., 2011; Graugnard et al., 2012), and that is also observed in our 0 → 30-d group (Chen et al., 2016). Nevertheless, the plasma creatinine level seems not explained by UHS in our cows.

In the current study, cows fed a lipogenic ration had greater cholesterol levels in plasma after calving compared with cows fed a glucogenic ration. In addition, cows with RU and fed a lipogenic ration had higher levels of albumin in plasma compared with cows with NRU and fed a lipogenic ration. A previous study showed that cows with a dietary fat supplementation of 5% (DM basis) in ration had increased plasma cholesterol levels due to higher synthesis of lipoproteins by the gut compared with cows not receiving fat supplementation (Carroll et al., 1990). Cholesterol is known as a marker of a negative APP (i.e., apolipoproteins) and also a precursor for steroid hormone synthesis and it

increases in plasma by feeding supplemental fat to dairy cows (Grummer and Carroll, 1991). Albumin is not only associated with fat infiltration in the liver, but also with uterine diseases in dairy cows (Bobe et al., 2004), and an important protein to transport long-chain fatty acids from adipocyte triacylglycerol stores to muscle tissue (Spector, 1986). Therefore, it is likely that high albumin levels in plasma in cows with RU and fed a lipogenic ration may be due to higher synthesis of lipoproteins by the gut and intense transport of fat in these cows, compared with cows with NRU and fed a lipogenic ration. In any case, the higher concentration of albumin in plasma during the peripartum reflects less inflammation of the cows and higher liver functionality (Bertoni et al., 2008; Trevisi et al., 2012).

The dry period length treatment in the current study was associated with a contrast in prepartum diets, prepartum antibiotic use at dry off, and twice daily milking regime before calving. This results in a system comparison among dry period strategies rather than specifically comparing dry period lengths only. This might imply that observed effects attributed to dry period length could be related to the associated contrasts in diet composition, antibiotic use, or milking regime among dry period length treatments. Earlier study has attempted to disentangle dry period length from the associated effect of a change in diet composition by feeding cows with a short dry period prepartum a lactation ration and comparing this with cows with no dry period (Rastani et al., 2005), resulting in an increase in EB and body condition prepartum for cows with 30-d dry period. Another study compared cows with no dry period with cows with 30-d dry period, but dried off without dry cow antibiotics (Van Hoeij et al., 2018). This resulted still in an increase in somatic cell count for cows after no dry period, compared with cows with a 30-d dry period. Although, the system comparison of dry period management strategies is sensible for practical and biological reasons, it makes it hard to identify specific effects of dry period length and possible confounding effects of the associated changes in diet, antibiotics or milking regime should be considered.

As we suggested earlier (Mayasari et al., 2017), the increased positive APP (ceruloplasmin) and ROM in plasma in early lactation could partly be explained by many factors such as dry period length, ration, EB, and occurrence of clinical health problems related to inflammation. In addition, from the current study, we conclude that increased inflammatory status in dairy cows in early lactation can be related to a nonrecovering uterus postpartum, independent of dry period length. This suggests that the inflammatory status attributed to NEB should be disentangled in various concurrently operating inflammatory responses. This implies that UHS being considered as one of the subclinical and clinical health problems related to inflammation may partly explain the changes in inflammatory biomarkers in early lactation.

## LITERATURE CITED


Ametaj, B. N., B. J. Bradford, G. Bobe, R. A. Nafikov, Y. Lu, J. W. Young, and D. C. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. Can. J. Anim. Sci. 85:165–175. doi:10.4141/a04-043

Ametaj, B. N., S. Iqbal, F. Selami, J. F. Odhiambo, Y. Wang, M. G. Gänzle, S. M. Dunn, and Q. Zebeli. 2014. Intravaginal administration of lactic acid bacteria modulated the incidence of purulent vaginal discharges, plasma haptoglobin concentrations, and milk production in dairy cows. Res. Vet. Sci. 96:365–370. doi:10.1016/j.rvsc.2014.02.007

Baumann, H., and J. Gauldie. 1994. The acute phase response. Immunol. Today 15:74–80. doi:10.1016/0167-5699(94)90137-6

Benzie, I. F. F. and J. J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochem. 239:70–76. doi:10.1006/abio.1996.0292

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91:3300–3310. doi:10.3168/jds.2008-0995

Bicalho, M. L. S., F. S. Lima, E. K. Ganda, C. Foditsch, E. B. S. Meira Jr, V. S. Machado, A. G. V. Teixeira, G. Oikonomou, R. O. Gilbert, and R. C. Bicalho. 2014. Effect of trace mineral supplementation on selected minerals, energy metabolites, oxidative stress, and immune parameters and its association with uterine diseases in dairy cattle. J. Dairy Sci. 97:4281–4295. doi:10.3168/jds.2013–7832

Bionaz, M., E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Bertoni. 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. J. Dairy Sci. 90:1740–1750. doi:10.3168/jds.2006-445

Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J. Dairy Sci. 87:3105–3124. doi:10.3168/jds.S0022-0302(04)73446-3

Burke, C. R., S. Meier, S. McDougall, C. Compton, M. Mitchell, and J. R. Roche. 2010. Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows. J. Dairy Sci. 93:5363–5373. doi:10.3168/jds.2010-3356

Calamari, L., A. Ferrari, A. Minuti, and E. Trevisi. 2016. Assessment of the main plasma parameters included in a metabolic profile of dairy cow based on fourier transform mid-infrared spectroscopy: preliminary results. BMC Vet. Res. 12:4. doi:10.1186/s12917-015-0621-4

Carroll, D. J., M. J. Jerred, R. R. Grummer, D. K. Combs, R. A. Pierson, and E. R. Hauser. 1990. Effects of fat supplementation and immature alfalfa to concentrate ratio on plasma progesterone, energy balance, and reproductive traits of dairy cattle. J. Dairy Sci. 73:2855–2863. doi:10.3168/jds.S0022-0302(90)78973–4

Chan, J. P., C. Chang, W. Hsu, W. Liu, and T. Chen. 2010. Association of increased serum acute-phase protein concentrations with reproductive performance in dairy cows with postpartum metritis. Vet. Clinical Pathol. 39:72–78. doi:10.1111/j.1939-165X.2009.00182.x

Chen, J., J. J. Gross, H. A. van Dorland, G. J. Remmelink, R. M. Bruckmaier, B. Kemp, and A. T. M. van Knegsel. 2015a. Effects of dry period length and dietary energy source on metabolic status and hepatic gene expression of dairy cows in early lactation. J. Dairy Sci. 98:1033–1045. doi:10.3168/jds.2014–8612

Chen, J., G. J. Remmelink, J. J. Gross, R. M. Bruckmaier, B. Kemp, and A. T. M. van Knegsel. 2016. Effects of dry period length and dietary energy source on milk yield, energy balance, and metabolic status of dairy cows over 2 consecutive years: effects in the second year. J. Dairy Sci. 99:4826–4838. doi:10.3168/jds.2015–10742

Chen, J., N. M. Soede, H. A. van Dorland, G. J. Remmelink, R. M. Bruckmaier, B. Kemp, and A. T. M. van Knegsel. 2015b. Relationship between metabolism and ovarian activity in dairy cows with different dry period lengths. Theriogenology 8:1387–1396. doi:10.1016/j.theriogenology.2015.07.025

Chen, J., N. M. Soede, G. J. Remmelink, R. M. Bruckmaier, B. Kemp, and A. T. M. van Knegsel. 2017. Relationships between uterine health and metabolism in dairy cows with different dry period lengths. Theriogenology 101:8–14. doi:10.1016/j.theriogenology.2017.06.017

CVB (Centraal Veevoederbureau). 2005. Veevoedertabel. Gegevens over chemische samenstelling, verteerbaarheid en voederwaarde van voedermiddelen. Lelystad (the Netherlands): CVB.

Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2010. Risk factors for postpartum uterine diseases in dairy cows. J. Dairy Sci. 93:5764–5771. doi:10.3168/jds.2010-3429

Feingold, K. R., R. A. Memon, A. H. Moser, and C. Grunfeld. 1998. Paraoxonase activity in the serum and hepatic mrna levels decrease during the acute phase response. Atherosclerosis 139:307–315. doi:10.1016/S0021-9150(98)00084-7

Ferré, N., J. Camps, E. Prats, E. Vilella, A. Paul, L. Figuera, and J. Joven. 2002. Serum paraoxonase activity: a new additional test for the improved evaluation of chronic liver damage. Clinical Chem. 48:261–268. [Accessed February 2002]. http://clinchem.aaccjnls.org/content/clinchem/48/2/261.full.pdf

de Feu, M. A., A. C. O. Evans, P. Lonergan, and S. T. Butler. 2009. The effect of dry period duration and dietary energy density on milk production, bioenergetic status, and postpartum ovarian function in Holstein-Friesian dairy cows. J. Dairy Sci. 92:6011–6022. doi:10.3168/jds.2009–2374

Fleck, A. 1989. Clinical and nutritional aspects of changes in acute-phase proteins during inflammation. Proc. Nutr. Soc. 48:347–354. doi:10.1079/PNS19890050

Gilbert, R. O., S. T. Shin, C. L. Guard, H. N. Erb, and M. Frajblat. 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology. 64:1879–1888. doi:10.1016/j.theriogenology.2005.04.022

Graugnard, D. E., M. Bionaz, E. Trevisi, K. M. Moyes, J. L. Salak-Johnson, R. L. Wallace, J. K. Drackley, G. Bertoni, and J. J. Loor. 2012. Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum1. J. Dairy Sci. 95:1749–1758. doi:10.3168/jds.2011–4579

Green, M. P., A. M. Ledgard, M. C. Berg, A. J. Peterson, and P. J. Back. 2009. Prevalence and identification of systemic markers of sub-clinical endometritis in postpartum dairy cows. In: Proceedings of the New Zealand Society of Animal Production. New Zealand Society of Animal Production. p. 37–42. ISSN: 0370-2731

Grummer, R. R., and D. J. Carroll. 1991. Effects of dietary fat on metabolic disorders and reproductive performance of dairy cattle. J. Anim. Sci. 69:3838–3852. doi:10.2527/1991.6993838x

Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff, and J. L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet. Immunol. and Immunopathol. 113:21–29. doi:10.1016/j.vetimm.2006.03.022

Hassanpour, A., M. Gholipour, and A. Rezaie. 2011. The effect of using monensin on haptoglobin serum level in dairy cattle. J. Animal Vet. Advanc. 10:1364–1367. doi:10.3923/javaa.2011.1364.1367

Huzzey, J. M., T. F. Duffield, S. J. LeBlanc, D. M. Veira, D. M. Weary, and M. A. von Keyserlingk. 2009. Short communication: haptoglobin as an early indicator of metritis. J. Dairy Sci. 92:621–625. doi:10.3168/jds.2008-1526

Jacometo, C. B., J. S. Osorio, M. Socha, M. N. Corrêa, F. Piccioli-Cappelli, E. Trevisi, and J. J. Loor. 2015. Maternal consumption of organic trace minerals alters calf systemic and neutrophil mrna and microRNA indicators of inflammation and oxidative stress. J. Dairy Sci. 98:7717–7729. doi:10.3168/jds.2015-9359

Janovick, N. A., Y. R. Boisclair, and J. K. Drackley. 2011. Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous holstein cows. J. Dairy Sci. 94:1385–1400. doi:10.3168/jds.2010-3303

Kaya, S., O. Merhan, C. Kacar, A. Colak, and K. Bozukluhan. 2016. Determination of ceruloplasmin, some other acute phase proteins, and biochemical parameters in cows with endometritis. Vet. World 9:1056–1062. doi:10.14202/vetworld.2016.1056-1062

Kizil, O., Y. Akar, M. Yuksel, and N. Saat. 2010. Oxidative stress in cows with acute puerperal metritis. Revue Méd. Vét. 161:353–357. https://www.revmedvet.com/2010/RMV161_353_357.pdf

LeBlanc, S., T. Duffield, K. Leslie, K. Bateman, G. P. Keefe, J. Walton, and W. Johnson. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J. Dairy Sci. 85:2223–2236. doi:10.3168/jds.S0022-0302(02)74302–6

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. Cary (NC): SAS Institute.

Magata, F., I. Morino, M. Teramura, E. Tsunoda, C. Kawashima, S. Haneda, A. Miyamoto, K. Kida, and

T. Shimizu. 2017. Impact of metritis on the generation of reactive oxygen species by circulating phagocytes and plasma lipopolysaccharide concentration in peripartum dairy cows. Anim. Sci. J. 88:248–253. doi:10.1111/asj.12642

Manimaran, A., A. Kumaresan, S. Jeyakumar, T. K. Mohanty, V. Sejian, N. Kumar, L. Sreela, M. A. Prakash, P. Mooventhan, A. Anantharaj, et al. 2016. Potential of acute phase proteins as predictor of postpartum uterine infections during transition period and its regulatory mechanism in dairy cattle. Vet. World 9:91–100. doi:10.14202/vetworld.2016.91-100

Mayasari, N., J. Chen, A. Ferrari, R. M. Bruckmaier, B. Kemp, H. K. Parmentier, A. T. M. van Knegsel, and E. Trevisi. 2017. Effects of dry period length and dietary energy source on inflammatory biomarkers and oxidative stress in dairy cows. J. Dairy Sci. 100:4961–4975. doi:10.3168/jds.2016-11857

Mordak, R. 2009. Postpartum serum concentration of haptoglobin in cows with fetal membranes retention. Cattle Pract. 17:100–102. http://www.bcva.org.uk

Moretti, P., M. Probo, N. Morandi, E. Trevisi, A. Ferrari, A. Minuti, M. Venturini, S. Paltrinieri, and A. Giordano. 2015. Early post-partum hematological changes in Holstein dairy cows with retained placenta. Anim. Reprod. Sci. 152:17–25. doi:10.1016/j.anireprosci.2014.11.019

Owen, J. A., F. C. Better, and J. Hoban. 1960. A simple method for the determination of serum haptoglobins. J. Clin. Pathol. 13:163–164. doi:10.1136/jcp.13.2.163

Pires, J. A., C. Delavaud, Y. Faulconnier, D. Pomiès, and Y. Chilliard. 2013. Effects of body condition score at calving on indicators of fat and protein mobilization of periparturient holstein-friesian cows. J. Dairy Sci. 96:6423–6439. doi:10.3168/jds.2013-6801

Pohl, A., O. Burfeind, and W. Heuwieser. 2015. The associations between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes, and metritis. J. Dairy Sci. 98:4544–4551. doi:10.3168/jds.2014-9181

Prunner, I., H. Pothmann, K. Wagener, M. Giuliodori, J. T. Huber, M. Ehling-Schulz, and M. Drillich. 2014. Dynamics of bacteriologic and cytologic changes in the uterus of postpartum dairy cows. Theriogenology. 82:1316–1322. doi:10.1016/j.theriogenology.2014.08.018

Rastani, R. R., R. R. Grummer, S. J. Bertics, A. Gümen, M. C. Wiltbank, D. G. Mashek, and M. C. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. J. Dairy Sci. 88:1004–1014. doi:10.3168/jds.S0022-0302(05)72768-5

Sattler, T., and M. Fürll. 2004. Creatine kinase and aspartate aminotransferase in cows as indicators for endometritis. J. Vet. Med. A. Physiol. Pathol. Clin. Med. 51:132–137. doi:10.1111/j.1439-0442.2004.00612.x

Schneider, A., M. N. Corrêa, and W. R. Butler. 2013. Short communication: acute phase proteins in Holstein cows diagnosed with uterine infection. Res. Vet. Sci. 95:269–271. doi:10.1016/j.rvsc.2013.02.010

Shukla, N., J. Maher, J. Masters, G. D. Angelini, and J. Y. Jeremy. 2006. Does oxidative stress change ceruloplasmin from a protective to a vasculopathic factor? Atherosclerosis 187:238–250. doi:10.1016/j.atherosclerosis.2005.11.035

Skinner, J. G., R. A. Brown, and L. Roberts. 1991. Bovine haptoglobin response in clinically defined field conditions. Vet. Rec. 128:147–149. doi:10.1136/vr.128.7.147

Spector, A. A. 1986. Structure and lipid binding properties of serum albumin. Methods Enzymol. 128:320–339. doi:10.1016/0076-6879(86)28077-5

Sunderman, F. W. and S. Nomoto. 1970. Measurement of human serum ceruloplasmin by its p-phenylenediamine oxidase activity. Clinical Chem. 16:903–910. http://clinchem.aaccjnls.org/content/clinchem/16/11/903.full.pdf

Tamminga, S., W. M. Van Straalen, A. P. J. Subnel, R. G. M. Meijer, A. Steg, C. J. G. Wever, and M. C. Blok. 1994. The Dutch protein evaluation system: the DVE/OEB system. Livest. Prod. Sci. 40:139–155. doi:10.1016/0301-6226(94)90043–4

Trevisi, E., M. Amadori, S. Cogrossi, E. Razzuoli, and G. Bertoni. 2012. Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Res. Vet. Sci. 93:695–704. doi:10.1016/j.rvsc.2011.11.008

Trevisi, E., A. R. Ferrari, and G. Bertoni. 2008. Productive and metabolic consequences induced by the retained placenta in dairy cows. Vet. Res. Commun. 32 (Suppl 1):S363–S366. doi:10.1007/s11259-008-9149-4

Van Es, A. J. H. 1975. Feed evaluation for dairy cows. Livest. Prod. Sci. 4:95–175. doi:10.1016/0301-6226 (75)90029-9

Van Hoeij, R. J., T. J. G. M. Lam, R. M. Bruckmaier, J. Dijkstra, G. J. Remmelink, B. Kemp, and A. T. M. van Knegsel. 2018. Udder health of dairy cows fed different dietary energy levels after a short or no dry period without use of dry cow antibiotics. J. Dairy Sci. 101:4570–4585. doi:10.3168/jds.2017-13448

Van Knegsel, A. T., G. J. Remmelink, S. Jorjong, V. Fievez, and B. Kemp. 2014. Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. J. Dairy Sci. 97:1499–1512. doi:10.3168/jds.2013-7391

Williams, E. J., D. P. Fischer, D. U. Pfeiffer, G. C. England, D. E. Noakes, H. Dobson, and I. M. Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63:102–117. doi:10.1016/j.theriogenology.2004.03.017