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Metabolic Load in Dairy Cows kept in Herbage based Feeding System and Suitability of potential Markers for compromised Well-being

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Summary

Herbage feeding with only little input of concentrates plays an important role in milk production in grassland dominated countries like Switzerland. The objective was to investigate effects of a solely herbage based diet and level of milk production on performance, and variables related to the metabolic, endocrine and inflammatory status to estimate the stress imposed on dairy cows. Twenty-five multiparous Holstein cows were divided into a control (C+, n=13) and a treatment group (C-, n=12), according to their previous lactation yield (4679-10808 kg) from week 3 ante partum until week 8 post partum (p.p.). While C+ received fresh herbage plus additional concentrate, no concentrate was fed to C- throughout the experiment. Within C+ and C-, the median of the preceding lactation yields (7752 kg) was used to split cows into a high- (HYC+, HYC-) and low-yielding (LYC+, LYC-) group. Throughout the study, HYC+ had a higher milk yield (35.9 kg/d) compared to the other subgroups (27.2-31.7 kg/d, $P < 0.05$). Plasma glucose (3.51 vs. 3.72 mmol/L) and IGF-1 (66.0 vs. 78.9 ng/mL) concentrations were lower in HYC-/LYC- compared to HYC+/LYC+ cows ($P < 0.05$). Plasma FFA and BHBA concentrations were dramatically elevated in HYC- (1.1 and 1.6 mmol/L) compared to all other subgroups (mean values: 0.5 and 0.6 mmol/L, $P < 0.05$). Saliva cortisol, plasma concentrations of serum amyloid A (SAA), haptoglobin (Hp), beta-endorphin (BE) and activity of alkaline phosphatase (AP) were not different between C+ and C-. In conclusion, herbage fed high-yielding cows without supplementary concentrate experienced a high metabolic load resulting in a reduced performance compared to cows of similar potential fed accordingly. Low-yielding cows performed well without concentrate supplementation. Interestingly, the selected markers for inflammation and stress such as cortisol, Hp, SAA, BE and AP gave no indication for the metabolic load being translated into compromised well-being.

Keywords: animal welfare, well-being, metabolism, herbage feeding, dairy cow

Metabolic Load in Dairy Cows kept in Herbage based Feeding Systems and Suitability of potential Markers for compromised Well-being

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Introduction

The topographic and climatic situation in alpine regions, including Switzerland favors a milk production system in which herbage feeding plays an important role to cover nutrient requirements of the dairy cow with little input of concentrates. During the last decades, milk yield in dairy cows increased worldwide and high-yielding cows are not an exception in higher reaches of the alps. However, a herbage dominated diet may have limitations with respect to the energy density and nutrient imbalances that could compromise dairy cow production, health and well-being, especially during early lactation when the cow is metabolically most challenged (Bruinenberg et al., 2002). Besides that, due to a changing quality of herbage across seasons, herbage-based feeding systems are likely to influence the metabolic status of dairy cows (Kaufmann et al., 2012). A high metabolic load is commonly observed in dairy cows during early lactation when the demands for energy, glucose, lipids, proteins, and minerals for milk synthesis cannot be covered by feed intake (Drackley, 1999; van Dorland et al., 2009; Gross et al., 2011a). Consequently, a transient negative energy balance (NEB) and nutrients during the postpartum period is inevitable and the dairy cow experiences a particular metabolic load which might also be considered as stress. A high level of metabolic load in early lactation may subsequently increase the risk for the occurrence of metabolic and related disorders, as some energetic processes (i.e. those maintaining general health) must be down-regulated (Knight et al., 1999) due to the high priority of the mammary gland.

Animal health contributes importantly to well-being and whenever an animal is diseased, its well-being is compromised (Bertoni et al., 2009; Barkema et al., 2015). With regard to dairy cow welfare, the major topics include lameness, mastitis, and metabolic disorders (Abeni and Bertoni, 2009; Barkema et al., 2015). A high metabolic load coming along with low plasma glucose concentrations and high plasma free fatty acids (FFA) and β-hydroxy-butyric acid (BHBA) concentrations is an obvious risk factor for various diseases (Reist et al., 2002; Hachenberg et al., 2007; Graber et al., 2012) and compromised immune response (Zarrin et al., 2014). Hence, the metabolic load itself may affect well-being as well. At present, most investigations tried to increase the understanding of the altered metabolic state and key nutrient demands during metabolic load commonly observed in early lactation or brought about by feed restrictions (Kolver and Muller, 1998; van Dorland et al., 2009; Gross et al., 2011a). To our knowledge, however, no study has been carried out that considered the metabolic state from a welfare perspective during a time-period such as early lactation when the cows' metabolic load is highest.

The main objective of the present study was to investigate the suitability of markers for inflammation and stress for discriminating different degrees of metabolic load which may in turn compromise the level of well-being in the high-yielding dairy cow kept in a herbage-based feeding system.

The hypothesis tested was that dairy cows kept on a fresh herbage based diet without concentrate undergo an extreme metabolic load during the periparturient period up to 8 wk p.p. which results in altered blood concentrations of markers for inflammation and stress thus indicating compromised health and well-being.

Material and Methods

Animals and Grouping

The experimental procedures followed the Swiss Federal Law on Animal Protection and were approved by the Committee of Animal Experiments of the Canton Fribourg, Switzerland. Twenty-five multiparous Holstein dairy cows (Parity: 3.2 ± 1.3; previous lactation yield: 4,679 to 10,808 kg) from the experimental herd of the Agroscope ILS research farm (Posieux, Switzerland) were involved in this study. Cows have been synchronized before being inseminated, and therefore all calvings occurred in May and June in the year of the experiment. Based on parity and previous lactation performance, cows were divided into two treatment groups. The two treatment groups involved one group (n = 12) that was fed a sole fresh herbage diet without concentrate (**C-**), and one group (control group, n = 13, **C+**) which received a fresh herbage diet balanced with additional concentrate according to the energy and nutrient requirements of the individual cow.

Housing, Feeding Regimen and Feed Analysis

The experiment was conducted in dairy cows from three weeks before the expected calving date up to the eighth week of lactation. Cows were adapted to herbage feeding on pasture during the dry period. The aim was to have the cows calving when sufficient herbage was available to be cut and fed indoors, which was supposed to be at the end of April.

All cows were housed in a free stall barn with free access to an outdoor paddock. Approximately five days before expected calving and up to three days thereafter, cows were kept in straw-bedded calving pens. After parturition, cows were milked twice daily in a milking parlor (at 05:30 and 16:30), and BW was measured automatically when cows were leaving the milking parlor. Fresh herbage was provided twice daily in feeding troughs connected to electronic balances for recording individual herbage intake (Insentec B.V., Marknesse, the Netherlands). While all cows of C+ and C- were fed herbage ad libitum, only cows of the C+ group received a weekly adapted amount of protein and energy rich concentrates (milk yield > 20 kg/d, for each kg more milk yield/d: +0.5 kg of additional concentrate, min. 2.0 kg, max. 7.0 kg/d) in transponder feeding stations to meet their energy and nutrient requirements (Agroscope, 2015). All cows were provided with mineral supplements (for the dry and lactation period, Table 1), and had free access to water. In addition, magnesium oxide (MgO) was provided to all animals in the first period of the experiment to avoid hypomagnesemia induced by feeding young herbage. Samples of freshly cut herbage were taken twice daily for determination of DM content and two pooled samples per week were analyzed for their crude nutrient content according to Thanner et al. (2014). Samples of the protein and energy rich concentrates were analyzed for their feeding value thrice during the course of the experiment. Content of energy and absorbable protein in the small intestine was estimated as described in Agroscope (2015). The chemical composition and nutrient values of herbage and concentrates provided are shown in Table 1. Energy balance (EB) for individual animals was calculated weekly as the difference between energy intake (based on feed analyses and

NEL estimation according to Agroscope (2015), and measured DMI per animal) and the energy output (requirements for maintenance and milk production).

Milk, Blood, and Saliva Sampling and Analysis

Milk samples from two consecutive milkings (evening and morning milk sample) were pooled proportionally once weekly and analyzed for fat, protein, and lactose content by FT-IR (Combi-Foss FT 6000, Foss, Hillerød, Denmark).

Blood sampling took place once weekly throughout the study between 06:00 AM and 08:30 AM after milking and before feeding. Blood was sampled into EDTA-coated, evacuated tubes (Vacuette, Greiner Bio-One, St. Gallen, Switzerland) by puncture of the jugular vein and samples were kept on wet ice. Plasma was harvested after centrifugation for 20 min at 2,500 × *g* and +4 °C, and thereafter stored at -20 °C until analysis. Saliva samples were taken with Salivette saliva collection tubes immediately after blood sampling in weeks -2, 2, 4, 6, and 8 relative to parturition. Until analysis, saliva samples were stored at -20 °C. Saliva cortisol concentration was determined with the Salimetrics Cortisol Enzyme Immunoassay Kit according to the manufacturer's protocol.

Concentrations of plasma glucose, FFA, BHBA, and IGF-1 were determined in weekly samples as described earlier by Gross et al. (2011a). For weeks -2, 3, and 8 relative to calving, plasma concentrations of Hp, SAA, AP, and BE were determined by commercially available kits (Hp: kit no. E-10HPT (bovine specific ELISA, obtained from Lucerna-Chem, Lucerne, Switzerland); SAA: kit no. MBS700691 (bovine specific ELISA; MyBioSource, Antwerpen, Belgium); AP: kit no. ALP IFCC (Diatools AG, Villmergen, Switzerland); BE: kit no. RK-022-06 (bovine specific RIA, Phoenix Europe GmbH, Karlsruhe, Germany). The standard curve for Hp ranged from 15.6 to 1,000 ng/mL, for SAA from 0.1 to 20 µg/mL, and for BE from 10 to 1,280 pg/mL. Standard solutions were provided by the manufacturer. Limits of detection were 7.8 ng/mL (Hp), 0.05 µg/mL (SAA), and 2 U/L (AP). Intra- and inter-assay precision was < 6% (Hp), < 15% (SAA), < 7% (BE), and < 2% (AP).

Metabolic and infectious Health Disorders

Before selecting the cows for the experiment, a medical check-up including vital parameters was performed. Cows were daily inspected for the development of metabolic and infectious diseases (ketosis, acidosis, milk fever, mastitis, lameness, and fertility disorders). Cows that showed obvious clinical signs of a health disorder were treated accordingly. After treatment, the recovered cows remained in the study. Drop out from the study was applied when the cow did not seem to recover in response to the treatment, or during repetitive occurrence of the same disorder. Due to severe downer cow syndrome and symptoms of nervous acetonemia, two cows of the C- group (8,488 and 10,150 kg milk yield in their previous lactation) were removed from the experimental trial in week 1 and 3 p.p., respectively.

Statistical Analysis

All data presented in tables and figures are means ± SEM, except where indicated as SD. Data were checked for normal distribution by the UNIVARIATE procedure in SAS (Version 9.4, SAS Institute, Cary, NC, USA). In cases of not being normally distributed, data were log-transformed for statistical evaluations. As one of the objectives of the present study was to evaluate the effects of milk production level on metabolic load, all measured plasma BHBA concentrations among all time-points of sampling were plotted against the previous lactation performance of cows (Figure 1). The threshold of 1.5 mmol/L for plasma BHBA concentration, which indicates subclinical ketosis in dairy cows (Heuer et al., 1999; Compton et al., 2015), was exceeded predominantly by cows above 7,600 kg milk yield. This production level was very close to the median of the lactational performance of the C+ and C- group. Thus, a high- (HY) and low- (LY) yielding subgroup within both, C+ and C-, was created by splitting cows at the respective median (7752 kg). For the evaluation of treatment effects and milk yield levels on performance, metabolic, and welfare-related parameters, a MIXED model was used including week, group [HYC+ (n = 6), LYC+ (n = 7), HYC- (n = 7), LYC- (n = 5)], parity, and the week by group interaction as fixed effects. The repeated subject was the individual cow. Group differences over time were detected by the Bonferroni t-test. A

1-sample binomial test was used to evaluate the differences between the occurrence of health disorders in treatment groups. P-values < 0.05 were considered to be significant.

Results

Feed intake, BW, and Energy Balance (EB)

Throughout the entire study, DMI was higher in HYC+ compared to the other groups (P < 0.05; Figure 2B). Dry matter intake increased from week 1 before parturition to a peak in week 7 p.p. for all groups (Figure 2B). Independent of lactational performance, cows receiving additional concentrate showed a higher DMI during lactation compared to animals without concentrate supplementation (P < 0.05; Figure 2B).

In all cows, BW declined after parturition (Figure 2C). From week 3 p.p. onwards, BW remained relatively constant in all experimental groups (Figure 2C).

Before parturition, no differences in EB between groups were detected (Figure 2D). In week 1 p.p., EB was more negative in HYC+ and HYC- compared to LYC+ and LYC- (P < 0.05; Figure 2D). While EB was not different among LYC+, LYC-, and HYC+ from week 2 to 8 p.p. (P > 0.05), EB was lower in HYC- compared to LYC+, LYC-, and HYC+ (P < 0.05; Figure 2D).

Milk yield and Milk composition

Milk yield was higher in HYC+ compared to HYC-, as well as in LYC+ compared to LYC- (P < 0.05, Figure 2A). While milk fat content decreased from week 1 to 8 p.p. in HYC+, LYC+, and LYC-, HYC- had a higher milk fat content from week 2 to 6 p.p. compared to the other groups (P < 0.05, data not shown). Milk protein content decreased continuously from week 1 to 8 p.p. (Data not shown). No differences between C+ and C- were observed in milk protein content (P > 0.05). While the milk fat to protein ratio was not different between HYC+, LYC+, and LYC- from weeks 1 to 6 p.p. (P > 0.05), HYC- showed a milk fat to protein ratio above 1.5 from weeks 2 to 6 p.p. (Figure 3A). While milk lactose content did not differ between

HYC+, LYC+, and LYC-, HYC- had a lower milk lactose content throughout the study ($P < 0.05$, Figure 3B).

Metabolic Variables

Figure 4A shows the plasma glucose concentration in the experimental groups. Plasma glucose concentration in all groups declined after parturition, and further decreased in HYC- until a nadir in week 4 p.p.. Glucose concentration in HYC- was lower compared to the other groups from week 3 to 8 p.p. ($P < 0.05$, Figure 4A). Plasma FFA concentration increased for all cows until week 2 p.p. and declined thereafter (Figure 4B). High yielding cows (HYC+, HYC-) had a higher plasma FFA concentration in week 1 p.p. compared to lower yielding cows (LYC+, LYC-; $P < 0.05$). From week 3 to 6 p.p., HYC- had higher plasma FFA concentrations compared to the other groups ($P < 0.05$, Figure 4B). While there were no differences between groups in plasma BHBA concentrations up to week 2 p.p., HYC- had higher BHBA concentrations from week 3 to 8 p.p. ($P < 0.05$, Figure 4C). Within one week, plasma concentrations of IGF-1 decreased from late gestation to lactation (Figure 4D). In week 1 p.p., no differences between groups were observed ($P > 0.05$). Though C+ and C- did not differ in their previous lactation performance, HYC- and LYC- had lower plasma IGF-1 concentrations compared to HYC+ and LYC+ from week 2 to 8 p.p. ($P < 0.05$, Figure 4D).

Animal Health

Two animals of the HYC- group were removed from the experiment in the very early stage of lactation due to severe downer cow syndrome and signs of nervous acetonemia, respectively. Considering these two cows, in total 21 cases (distributed on 11 cows) of health disorders requiring a veterinary treatment were recorded during the experiment. Two cows showed signs of acetonemia (both in HYC-), one was anoestric (LYC+), two cows had diarrhea (both in LYC+), two cows were diagnosed having endometritis (both in HYC+), and two cows showed downer cow syndrome (both in HYC-). Claw problems occurred in two animals (HYC+ and LYC+), four cows were treated for clinical mastitis (one HYC-, two LYC-, one HYC+), one cow for pneumonia and fever (LYC-), and two cases for retained placenta (LYC+, HYC-). One cow (HYC-) experienced four health disorders during the trial (acute and chronic mastitis, acetonemia, and cases of retained placenta). Due to the low number of animals per group, the comparison of the occurrence of health disorders between groups must not be over-interpreted though in cows of HYC- more health disorders (4 out of 7 cows) were detected compared to HYC+, LYC+, and LYC- ($P < 0.05$).

Inflammatory and Stress related Variables

Throughout the study, no differences in saliva cortisol concentration between groups were observed ($P > 0.05$, Figure 5). Neither AP and Hp, nor concentrations of SAA and BE showed differences between groups ($P > 0.05$, Table 2).

Discussion

Currently, welfare in dairy cows is monitored by scoring different animal-based variables (e.g., percentage of lame and lean cows, access and comfort of feeding and resting facilities, etc.) and behavioral observations (De Vries et al., 2013; De Rosa et al., 2015). However, assessing animal status by these methods enables welfare classification mostly by categories at a herd level, but not for the individual cow. Though, a constrained well-being must be detected as early as possible in individual animals to maintain health and performance.

In Switzerland as well as in other countries favoring cultivation of grassland, herbage represents the most cost-effective forage whereas concentrates in particular if imported are quite expensive. In addition, herbage feeding systems meet the consumers' image of a safe and animal-friendly milk production. The present study investigated the nutritional flexibility of the Swiss high-yielding cow in a herbage based feeding system potentially affecting well-being and incidence of disease.

In the present study, herbage feeding without concentrate limited milk production to a level of 30 kg/d, while high-yielding cows reached an average milk yield of 40 kg/d at their peak of lactation. This finding is in agreement with an earlier study of Kolver and Muller (1998), who recorded milk production of 29.6 kg/d from cows grazing top quality pasture and 44.1 kg/d from similar cows fed a well-designed total mixed ration. Though animals in our study in both, the concentrate supplemented and only herbage fed group had a similar milk production potential, sole herbage feeding provided less nutrients compared to the control group. In the study of Haiger and Knaus (2011), a milk yield of 7500 kg during an entire lactation was observed without feeding concentrate, but was accompanied with pronounced mobilization of body reserves during the first third of lactation. In the study of Kolver and Muller (1998), milk production of cows on pasture was observed to be first-limited by energy intake, which was in part explained by a lower DMI on pasture compared to TMR feeding. Nonetheless, it was concluded that high nutrient intake from pasture can be achieved, but a marked loss of body condition was observed to maintain milk production, and supplemental energy was needed for milk production greater than 30 kg/d. It should be stressed that the study by Kolver and Muller (1998) was carried out with high-yielding dairy cows in their eighth week of lactation, when milk production is past the peak of lactation. While milk production increases despite a distinct NEB in early lactation indicating a favored nutrient direction towards the mammary gland, a similar NEB later in lactation caused an immediate decline in milk yield (Gross et al., 2011a). Despite ad libitum access to herbage, total DMI was significantly higher in cows supplemented with additional concentrate and thus providing more nutrients for milk production. Sehested et al. (2003) demonstrated that long-term omission of concentrate reduced milk production from 6723 kg energy corrected milk (ECM) per cow year in dairy cows fed with concentrate to 5090 kg ECM per cow year in cows fed without concentrate. Furthermore, they observed no indications of health problems associated with the reduced feeding level. Also in Haiger and Sölkner (1995), no differences were observed for veterinary costs, insemination rate, and days open between cows fed additional concentrate or not.

As observed by Gross et al. (2011a, b), undernutrition at 100 days in milk caused a reduction milk yield, thus supporting health. However, in early lactation, undernutrition increased metabolic load and compromised health of the dairy cows whereas milk production is maintained and increasing until disease (Kolver and Muller, 1998; Loor et al., 2007). To ensure animal well-being in general, diets provided must meet the animals' nutritional requirements depending on its physiological state as well as to minimize metabolic and nutritional disorders. In the present study, the sole herbage feeding caused an impressive metabolic load as evidenced by elevated plasma concentrations of NEFA and BHBA, in particular for the high-yielding dairy cows. The milk fat to protein-ratio was shown to be associated with the risk of an energy deficiency as commonly milk fat increases with concomitantly decreasing milk protein content (Heuer et al., 1999). In the present study, the milk fat to protein-ratio clearly reflected the enhanced energy deficiency in non-supplemented cows compared to concentrate fed cows. In agreement with earlier studies by Heuer et al. (1999) and Gross et al. (2011a), the milk fat to protein ratio exceeded similar thresholds (> 1.5) in the present study. Whereas low-yielding animals and adequately fed high-yielding cows showed only a temporary increase in plasma FFA and BHBA concentrations during the first three weeks after parturition in agreement with earlier findings of Gross et al. (2011a), particularly high-yielding cows without supplementary concentrate faced a prolonged elevation in plasma FFA and BHBA concentration exceeding levels for diagnosis of subclinical and clinical ketosis, respectively (Compton et al., 2015; Itle et al., 2015). Despite a limited energy output via milk production observed in the present study, high-yielding cows are very likely to preferentially partition nutrients towards the metabolically prioritized mammary gland during early lactation (Gross et al., 2015) and adapt to the prolonged NEB by a higher degree of the uncoupling their somatotropic axis. Grala et al. (2011) and Gross et al. (2015) showed that the performance level is related to the plasma concentrations of IGF-1 within the same breed. Though FFA derived from body fat mobilization and BHBA serve as energy source in metabolism and immune system (Zarrin et al., 2014), recent findings of Zarrin et al. (2013) showed an inhibitory effect of elevated BHBA concentrations on gluconeogenesis in mid-lactating dairy cows. Especially during early lactation, when plasma glucose concentration and rate of gluconeogenesis are low (Hammon et al., 2009; Aschenbach et al., 2010), an additional depression of gluconeogenesis by BHBA as shown for mid-lactating dairy cows might be assumed and is undesirable. Furthermore, elevated concentrations of circulating ketone bodies were shown to depress feed intake (Laeger et al., 2010; Laeger et al., 2012; Derno et al., 2013) and reproductive performance (Castro et al., 2012). However, high-yielding cows without supplementation of concentrate showed already a reduced DMI and a more negative EB compared to cows fed with additional concentrate.

The detrimental effects of a higher metabolic load on animal health in the present study might be related to the numerically increased number of health disorders requiring a veterinary treatment in high-yielding cows without concentrate supplementation. However, due to the low number of animals per group these results must not be over-interpreted. Nevertheless there is a need for adequate nutrition in high genetic merit cows to achieve sufficient animal health and good performance.

Biochemical markers in blood plasma and saliva established to assess animal well-being and stress were evaluated to characterize and understand the response towards the induced metabolic load by the experimental treatment. Markers of interest included cortisol, endogenous opioid peptides (beta-endorphin concentration), alkaline phosphatase (AP), and acute phase proteins (haptoglobin, serum amyloid A).

Cortisol is an important glucocorticoid secreted from the adrenal cortex. Its concentration in blood is commonly used as a stress indicator. In our study, cortisol levels were measured in saliva from each cow individually, which represents a non-invasive method that avoids causing additional stress, and thereby affecting cortisol levels in the animals. In the present study, salivary cortisol concentration was not affected by treatment and lactational stage. Trevisi et al. (2005) observed that basal plasma cortisol of dairy cows in commercial herds seemed linked to chronic stress in less well managed herds and therefore to the well-being of the cows. However, Trevisi et al. (2005) did not exactly define the differences between herds, management and feeding conditions. Apart from being an indicator of stress, cortisol is an important regulator of glucose in ruminants, which acts to increase hepatic gluconeogenesis (McDowell, 1983), and may therefore play an important role to overcome the metabolic load in early lactation. In the present study, saliva cortisol concentration did not reflect metabolic load in dairy cows. Furthermore, with respect to metabolic and infectious diseases, the role of cortisol is not fully understood yet (Forslund et al., 2010).

Endogenous opioid peptides are involved in many responses to stress (Bruckmaier et al., 1993; Pierzchała-Koziec et al., 1996), regulate various endocrine systems, including the hypothalamic-pituitary-adrenocortical (HPA) axis. Beta-endorphin is an endogenous opioid, which is produced by the pituitary gland and the hypothalamus, and is involved in the regulation of feed intake, and therefore impacts energy balance in ruminants (McShane et al., 1993). Acute stress (i.e., isolation, exercise, unfamiliar surrounding) may increase serum concentrations of beta-endorphin (Bruckmaier et al., 1993; Hashizume et al., 1994). Furthermore, endorphins are known to produce a feeling of well-being (Koneru et al., 2009). However, the unaffected BE concentrations in the present study do not indicate a constrained animal well-being nor stress induced by the experimental omission of concentrate. Consequently the herbage based feeding strategy in the present study might potentially not provoke acute stress in dairy cows compared to physically or mentally stressful changes in the environment.

Alkaline phosphatase activity in plasma of cattle has been often used as a stress marker in heat stressed cattle (Ronchi et al., 1999; Abeni et al., 2007). However, AP also seems related to daily gain, feed intake and feed utilization in growing cattle (Kunkel et al., 1953). Dairy cows in the present study had a lower plasma AP activity during early lactation compared to late gestation. Interestingly, AP activity was not affected by level of milk production and treatment. Contrary to our findings involving underfed dairy cows, plasma AP activity increased in feed restricted heifers in a thermoneutral environment, and was associated with an increased liver activity as metabolic adaptation in response to the reduced dry matter intake of the animals (Ronchi et al., 1999).

Acute phase proteins and cytokines are well acknowledged as inflammatory markers. Several studies (Lomborg et al., 2008; Saco et al., 2008) have shown that SAA and haptoglobin may serve as markers of acute stress provided by transportation and unfamiliar surroundings in adult cattle. Besedovsky et al. (1986) observed that a close relationship between the immune system and the HPA axis exists. Though dairy cows in the present study did not experience acute stress affecting Hp and SAA concentrations directly, the concomitant enhanced NEB in cows without supplementary concentrate feeding might provoke the risk of infectious diseases occurring in the early stage of lactation.

Conclusions

Herbage dominated feeding systems without supplementary concentrate cause a particularly high metabolic load in high-yielding dairy cows during early lactation. This in turn leads to a reduced lactational performance compared to cows of a similar potential fed according to their needs. Low-yielding dairy cows can perform well without concentrate supplementation. Interestingly, the enhanced metabolic load which we expected to be associated with reduced well-being was not indicated by markers related to inflammation and stress.

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Figure captions.

Figure 1.

Relationship between performance in the previous lactation and plasma beta-hydroxybutyrate (BHBA) concentration in concentrate supplemented (C+) and only herbage fed (C-) dairy cows. A threshold of 1.5 mmol/L was set for discussion of subclinical/clinical ketosis. The vertical dashed line represents the median of the lactational performance to distinguish between high- and low-yielding cows.

Figure 2.

Milk yield (A), dry matter intake (DMI, B), body weight (BW, C), and energy balance (D) in high-yielding cows with concentrate (HYC+), high-yielding cows without concentrate (HYC-), low-yielding cows with concentrate (LYC+), and low-yielding cows without supplementary concentrate (LYC-) during the experiment. Data are given as means ± SEM. Effects of parity, group (HYC+, HYC-, LYC+, LYC-), week relative to parturition, and group × week relative to parturition were considered significant at $P < 0.05$.

Figure 3.

Milk fat to protein ratio (A), and milk lactose content (B) in high-yielding cows with concentrate (HYC+), high-yielding cows without concentrate (HYC-), low-yielding cows with concentrate (LYC+), and low-yielding cows without supplementary concentrate (LYC-) during the experiment. Data are given as means ± SEM. Effects of parity, group (HYC+, HYC-, LYC+, LYC-), week relative to parturition, and group × week relative to parturition were considered significant at P < 0.05.

Figure 4.

Plasma concentrations of glucose (A), free fatty acids (FFA, B), beta-hydroxybutyrate (BHBA, C), and insulin-like growth factor-1 (IGF-1, D) in high-yielding cows with concentrate (HYC+), high-yielding cows without concentrate (HYC-), low-yielding cows with concentrate

(LYC+), and low-yielding cows without supplementary concentrate (LYC-) during the experiment. Data are given as means ± SEM. Effects of parity, group (HYC+, HYC-, LYC+, LYC-), week relative to parturition, and group × week relative to parturition were considered significant at $P < 0.05$.

Figure 5.

Saliva cortisol concentration in high-yielding cows with concentrate (HYC+), high-yielding cows without concentrate (HYC-), low-yielding cows with concentrate (LYC+), and low-yielding cows without supplementary concentrate (LYC-) during the experiment. Data are given as means ± SEM. Effects of parity, group (HYC+, HYC-, LYC+, LYC-), week relative to parturition, and group × week relative to parturition were considered significant at $P < 0.05$.

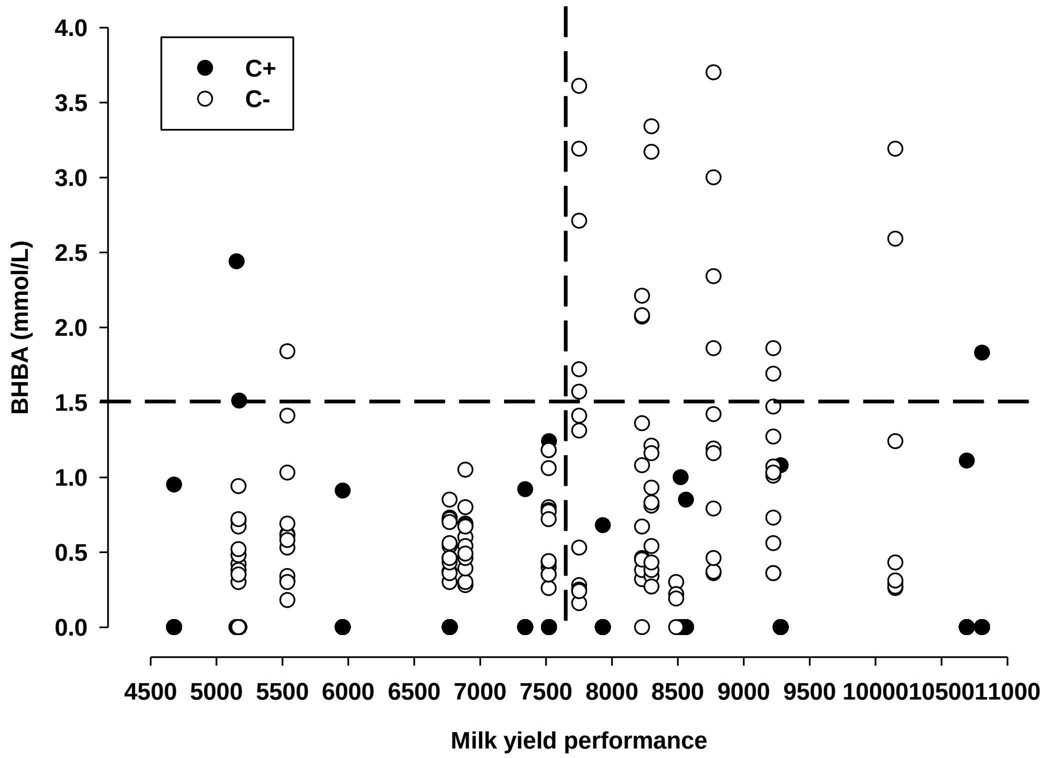

Figure 1.

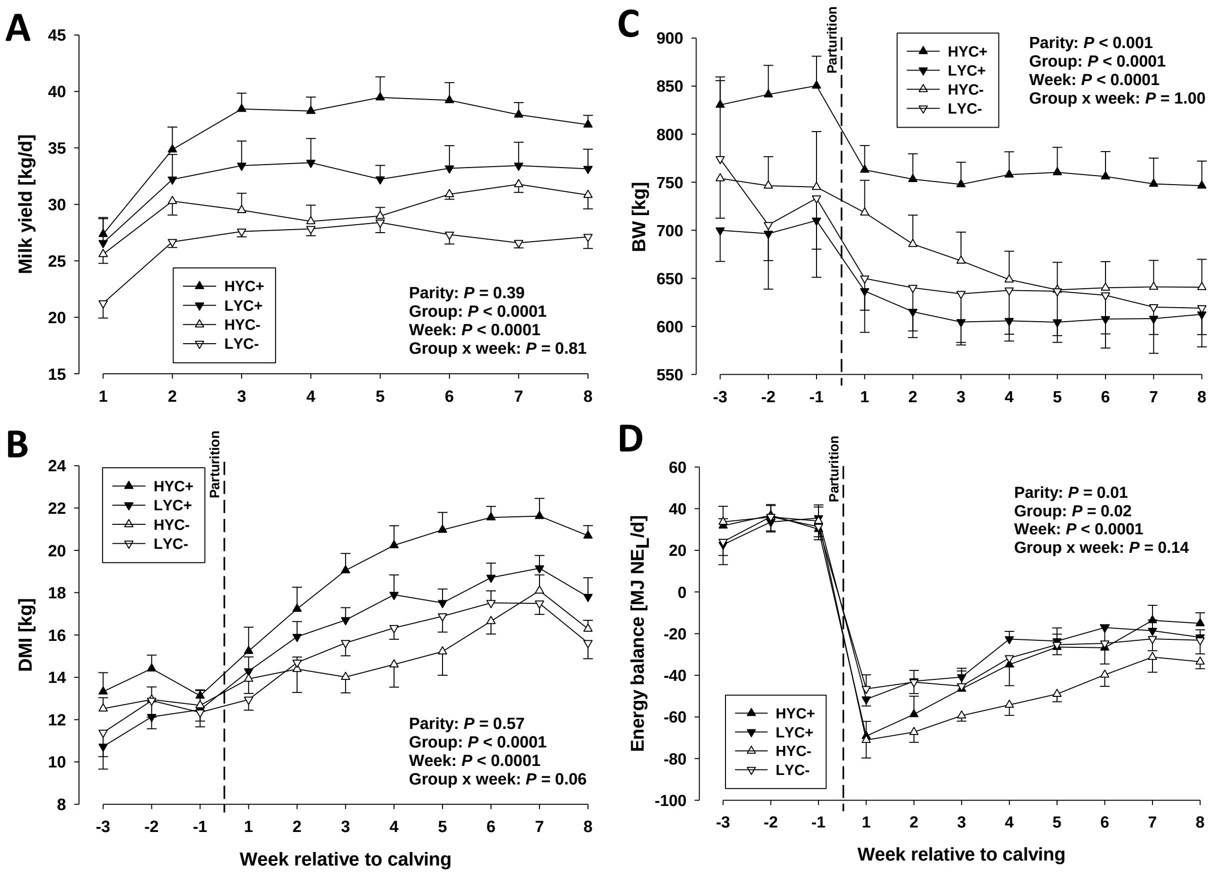

Figure 2.

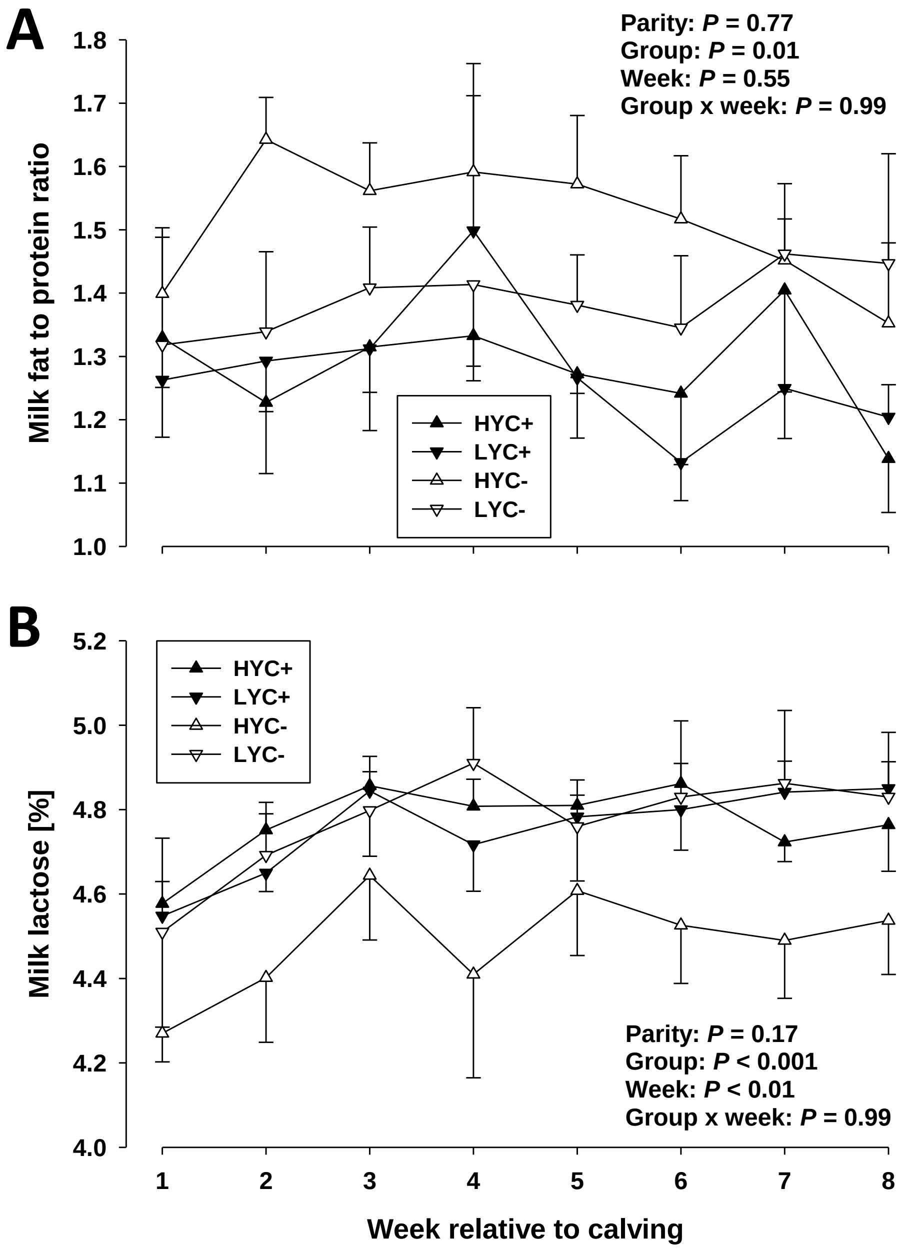
A
Parity: P = 0.77
Group: P = 0.01
Week: P = 0.55
Group x week: P = 0.99
Milk fat to protein ratio
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
HYC+
LYC+
HYC-
LYC-
B
5.2
5.0
4.8
4.6
4.4
4.2
4.0
Milk lactose [%]
HYC+
LYC+
HYC-
LYC-
Parity: P = 0.17
Group: P < 0.001
Week: P < 0.01
Group x week: P = 0.99
1
2
3
4
5
6
7
8
Week relative to calving

Figure 3.

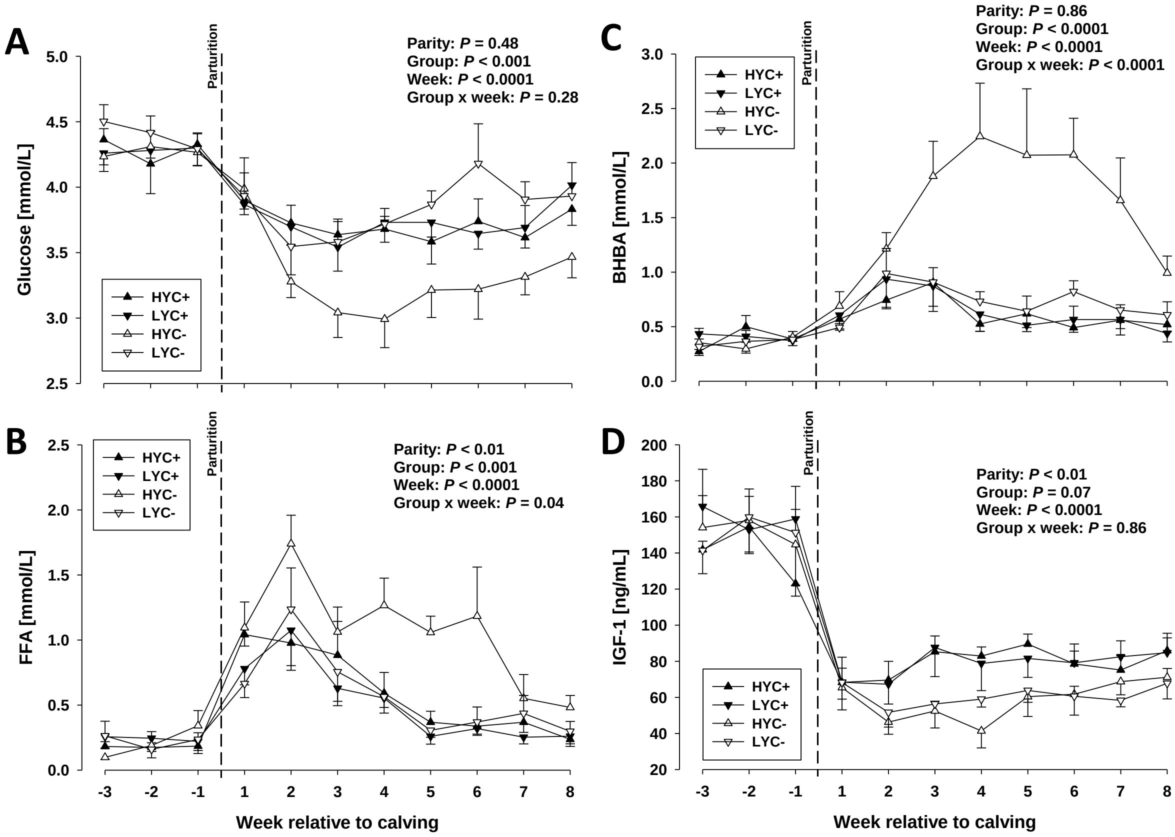
A
Parity: P = 0.48
Group: P < 0.001
Week: P < 0.0001
Group x week: P = 0.28
Parturition
Glucose [mmol/L]
HYC+
LYC+
HYC-
LYC-
B
Parity: P < 0.01
Group: P < 0.001
Week: P < 0.0001
Group x week: P = 0.04
FFA [mmol/L]
Week relative to calving
C
Parity: P = 0.86
Group: P < 0.0001
Week: P < 0.0001
Group x week: P < 0.0001
BHBA [mmol/L]
D
Parity: P < 0.01
Group: P = 0.07
Week: P < 0.0001
Group x week: P = 0.86
IGF-1 [ng/mL]
Week relative to calving

Figure 4.

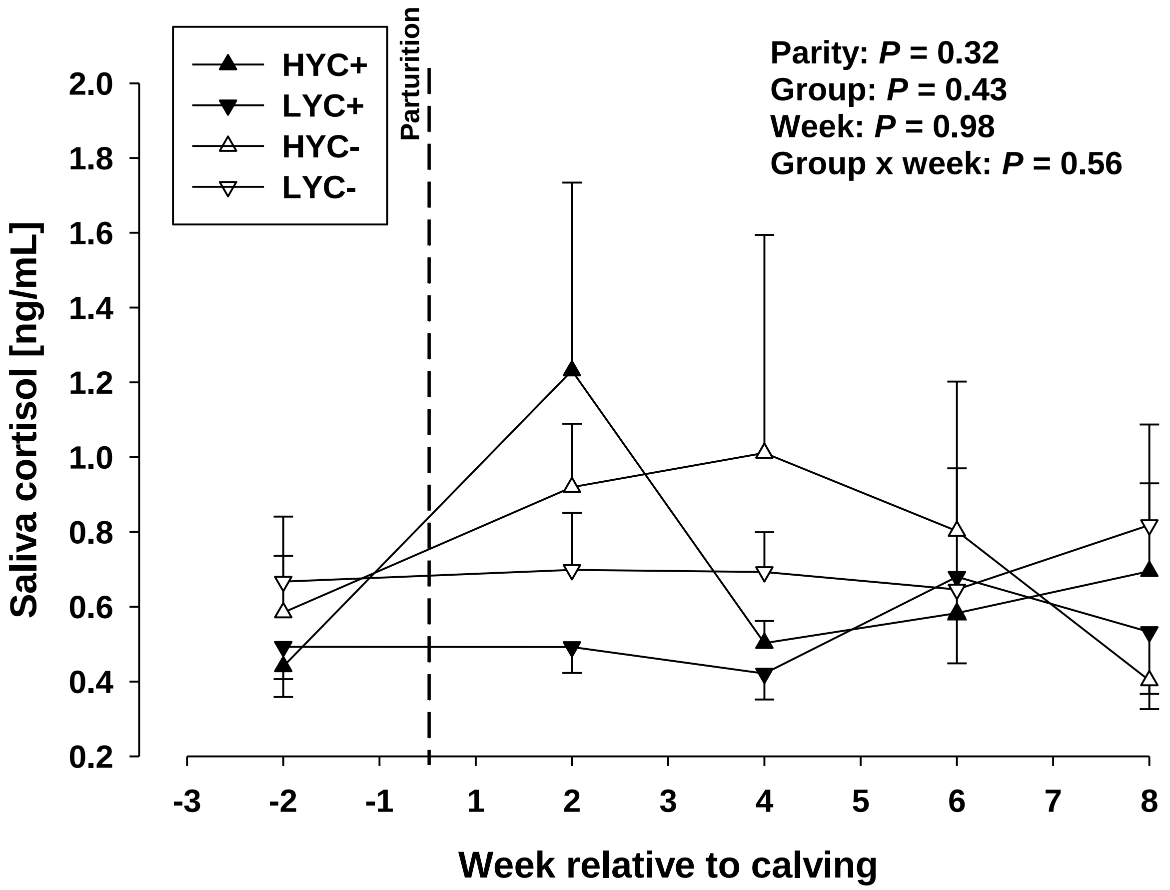
HYC+
LYC+
HYC-
LYC-
Parturition
Parity: P = 0.32
Group: P = 0.43
Week: P = 0.98
Group x week: P = 0.56
Saliva cortisol [ng/mL]
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
-3
-2
-1
1
2
3
4
5
6
7
8
Week relative to calving

Figure 5.

Table 1. Chemical composition and nutrient values of herbage (n = 36 samples analyzed) and concentrates (n = 3, each) provided during the experiment. Data are given as means ± SD.

	Herbage	Protein-rich concentrate (2.0 kg FM/d per cow)	Energy-rich concentrate (up to 5.0 kg FM/d per cow)
DM (g/kg of wet weight)	172 ± 28	887 ± 5	876 ± 6
Analyzed nutrient and mineral composition (g/kg DM)			
Crude ash	96.1 ± 11.6	53.4 ± 15.7	45.6 ± 2.1
CP	145 ± 31	248 ± 29	123 ± 14
NDF	457 ± 33	216 ± 139	251 ± 170
ADF	289 ± 21	61 ± 5	51 ± 2
WSC	179 ± 45	nd	nd
Starch	nd	500 ± 26	626 ± 8
Ca	6.59 ± 2.53	10.19± 4.45	8.26 ± 0.43
P	4.23 ± 0.80	5.25 ± 2.34	3.48 ± 0.29
Mg	1.76 ± 0.52	1.38 ± 0.37	1.18 ± 0.09
K	32.88 ± 3.93	5.07 ± 1.10	5.77 ± 0.53
Calculated energy and protein supply per kg DM			
NE_L (MJ)	5.75 ± 0.49	8.06 ± 0.14	8.11 ± 0.12
Absorbable protein at the duodenum (g)	95.3 ± 9.0	189.1 ± 15.6	81.4 ± 7.2

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC. Water soluble carbohydrates; NE_L, net energy lactation; nd, not determined.

Composition of mineral supplements for dry cows (in g/kg DM): Ca: 3.13 ± 0.63, P: 7.91 ± 0.25, Mg: 14.89 ± 1.73, K: 10.43 ± 0.48, Na: 47.54 ± 5.28.

Composition of mineral supplements for lactating cows (in g/kg DM): Ca: 107.67 ± 4.81, P: 52.12 ± 5.22, Mg: 19.53 ± 3.42, K: 3.28 ± 0.28, Na: 63.93 ± 6.23.

Table 2. Activity of alkaline phosphatase (AP), and concentrations of haptoglobin (Hp), serum-amyloid A (SAA), and beta-endorphin (BE) in high-yielding cows with concentrate (HYC+), high-yielding cows without concentrate (HYC-), low-yielding cows with concentrate (LYC+), and low-yielding cows without supplementary concentrate (LYC-) during the experiment. Data are given as means ± SEM. Effects of parity, group (HYC+, HYC-, LYC+, LYC-), week relative to parturition, and group × week relative to parturition were considered significant at P < 0.05.

Parameter	Group	Week relative to parturition			P-values			
		-2 a.p.	+3 p.p.	+8 p.p.	Parity	Group	Week	Group × week
AP (U/L)					P < 0.05	P = 0.61	P < 0.0001	P = 0.52
	HYC+	65.7 ± 13.6	38.3 ± 3.5	35.8 ± 4.9				
	LYC+	69.0 ± 6.3	40.9 ± 3.2	41.3 ± 2.9				
	HYC-	53.2 ± 10.3	33.8 ± 3.3	29.4 ± 1.7				
	LYC-	80.8 ± 14.6	39.2 ± 5.0	39.0 ± 3.2				
Hp (ng/mL)					P = 0.47	P = 0.79	P = 0.45	P = 0.99
	HYC+	513 ± 211	1,099 ± 604	1,163 ± 449				
	LYC+	408 ± 52	778 ± 256	810 ± 256				
	HYC-	393 ± 71	454 ± 10	654 ± 207				
	LYC-	461 ± 119	1,416 ±799	1,836 ± 1,286				
SAA (µg/mL)					P < 0.05	P = 0.13	P < 0.01	P < 0.05
	HYC+	0.96 ± 0.15	0.48 ± 0.03	0.45 ± 0.03				
	LYC+	0.74 ± 0.10	0.43 ± 0.03	0.45 ± 0.02				
	HYC-	0.77 ± 0.12	0.75 ± 0.23	1.12 ± 0.53				
	LYC-	1.10 ± 0.40	0.54 ± 0.06	0.57 ± 0.08				
BE (pg/mL)					P = 0.58	P = 0.56	P < 0.0001	P = 0.74
	HYC+	24.0 ± 4.5	25.5 ± 3.3	46.9 ± 6.7				
	LYC+	24.0 ± 3.8	20.7 ± 2.2	37.2 ± 8.0				
	HYC-	17.7 ± 2.1	24.6 ± 3.5	51.2 ± 11.5				
	LYC-	16.4 ± 1.8	29.7 ± 6.9	47.7 ± 9.9				

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