

Blood biochemical parameters measured during the periparturient period in cows of Holstein and Fleckvieh breeds differing in production purpose

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Abstract: The aim of this study was to compare the metabolic status of dairy and dual-purpose cows kept in a single herd under identical management conditions. Milk yield and blood biochemical parameters were examined during the periparturient period in Holstein (H) and Fleckvieh (F) cows. Blood samples were first taken on average 14 days prior to the expected date of calving, next in the periparturient period (1–4 days postpartum), and then at weekly intervals (together with milk samples) until the end of the experiment (8 weeks postpartum). Milk yields were higher in H cows from the second week after calving whereas milk protein content always was higher in F cows over the whole experimental period. The level of metabolic stress determined using blood concentrations of non-esterified fatty acids (NEFA), β -hydroxybutyrate (BHB), triglycerides, and cholesterol was similar in both breeds. The proportion of animals with concentrations of NEFA and BHB above thresholds indicating increased risk of negative energy balance and subclinical ketosis was higher in the H breed in the first 2 weeks after calving. This corresponds with numerically higher concentrations of NEFA and BHB in H cows. Changes in the protein status of animals generally reflected the development in energy metabolism parameters. Serum total protein, albumin, and urea levels were similar in the two breeds. Total globulin was higher in H cows than in F cows in weeks 3, 4, and 5 after calving, and the albumin-to-globulin ratio was lower in H cows than in F cows in weeks 2, 3, and 4 after calving. In conclusion, although the onset of changes in key metabolic parameters was rather faster and more pronounced in the H breed, similar dynamics in the development of these parameters indicated similar levels of adaptive performance and body energy mobilization processes in the two breeds.

Keywords: breed; dairy cattle; energy profile; periparturient period; protein status

Increased milk production is associated with a higher incidence of health problems and reduced reproductive performance in cows. The highest incidence of diseases occurs in the postpartum period and early lactation, when high-producing cows cannot meet the elevated energy requirements

imposed by the onset of lactation accompanied by reduced feed intake. This leads to negative energy balance (NEB) (Roche et al. 2013). Therefore, breeders aim to recognize the upcoming problem and to take preventive actions. One possible diagnostic tool available for this purpose is to evaluate

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an animal's metabolic profile through measuring blood parameters.

The commonly used indicators of energy profile and nutrient status in early-lactation cows are non-esterified fatty acids (NEFA), β -hydroxybutyrate (BHB), triglycerides (TG), and cholesterol concentrations (Puppel and Kuczynska 2016). Serum NEFA concentrations reflect the level of lipid reserve mobilization to compensate for the imbalance between nutrients consumed by the cow and nutrients secreted in milk. Thus, increased NEFA concentrations are associated with higher incidence of metabolic diseases (Gross et al. 2013). Although circulating NEFA can be oxidized in the hepatocytes or exported as constituents of very low-density lipoprotein (VLDL), postpartum release of NEFA from adipose tissue mostly exceeds liver oxidation capabilities and causes formation of ketones (BHB) and reesterification to TG (Drackley et al. 2001). Increased serum BHB concentrations may therefore indicate ketosis (McArt et al. 2013), whereas decreased serum TG levels are associated with lipolysis and the development of fatty liver syndrome (Drackley et al. 2001). Cholesterol, a component of VLDL, is closely related to NEFA metabolism. In early lactation, cholesterol levels increase physiologically to be available for VLDL synthesis and export, thereby naturally preventing fatty liver syndrome (Gross et al. 2015).

The blood parameters used to evaluate the protein status are total protein (TP), albumin, globulin, and urea (Puppel and Kuczynska 2016). Total serum protein is composed of albumin and globulins, and it provides background information about protein biosynthesis, utilization, and excretion, as well as renal failure, liver damage, and nutritional health. Urea is a degradation product of protein metabolism and is synthesized in the liver urea cycle. A high serum urea level may indicate increased ammonia detoxification and can be considered a risk factor for lipomobilization (Park et al. 2010).

Under certain circumstances it may be suitable to keep dairy and dual-purpose breeds in the same management conditions (Barth et al. 2011). However, the impact of identical management, and especially feeding, on the metabolic status of cows with significantly different milk yields may manifest itself in various health disorders. The concentrations of NEFA seem to be higher in high-yielding dairy cows than in dual-purpose breeds (Quiroz-Rocha et al. 2009). In contrast, Urdl et al. (2015) did not find any

differences in NEFA concentrations between dairy and dual-purpose breeds. Also, Barth et al. (2011) reported no differences in their study that, to our knowledge, is the only one comparing breeds under the same management conditions. The number of studies comparing the metabolic status of dual-purpose and dairy breed cows in early lactation remains limited, and especially when the breeds are managed in identical conditions. Moreover, the results of such studies that have been conducted are not consistent. The aim of the present study, therefore, was to compare selected serum parameters measured during the periparturient period in dairy Holstein (H) and dual-purpose Fleckvieh (F) cows kept under identical management conditions.

MATERIAL AND METHODS

Animals, diets and experimental design

Experimental procedures were approved by the Animal Care Committee of the Ministry of Agriculture of the Czech Republic. The study was conducted from November 2016 to May 2017 at the experimental station of the Institute of Animal Science in Prague, Czech Republic. The cows used in the experiment were selected from a herd of approximately 200 dairy cows of H and F breeds kept together under identical management conditions. Average milk yield and composition of the base herd during the experimental period are reported in Table 1. The herd was free from infectious and invasive diseases. Fresh cows were kept individually in straw-bedded box stalls until 7 days after calving and their health status was checked daily. They were then moved to straw-bedded freestalls with concrete-floor alleys. The barn was divided into five sections according to cow lactation phase and reproduction cycle (with sections for early-, mid- and late-lactation cows, as well as far-off and close-up dry cows).

Table 1. Average milk yields and composition of the base herd during the experimental period

Milk and components yields	Fleckvieh	Holstein
Milk (kg/305 days)	7 908	9 894
Fat (%)	3.87	3.47
Protein (%)	3.50	3.20

Table 2. Number and parity of Fleckvieh and Holstein cows included in the experiment

Parity	Fleckvieh	Holstein
1	6	17
2 and 3	8	14
4 and above	10	11
Total	24	42

A total of 66 primiparous and multiparous (up to lactation 9) cows were included in the experiment (Table 2). The experimental period began 14 days prior to the expected date of calving and continued until 8 weeks postpartum. The cows were milked twice daily (04:00 and 16:00). Milk production data were automatically recorded during each milking using the AfiFarm™ herd management software (SAE Afikim, Israel). Cows were fed *ad libitum* twice a day (04:00 and 16:00) with a total mixed ration (TMR) differing in its composition according to the lactation and reproduction cycle phase. The TMR was pushed up to the cows 6 times a day by an automatic feed pusher to ensure consistent access to feed. Close-up dry cows were fed a close-up diet starting 3 weeks prior to the expected date of calving. After calving, cows were switched to an early-lactation diet to meet the energy demands for high milk production (approximately 40 l per day). The TMRs were based on corn silage, alfalfa haylage, hay, wheat straw, concentrates, and mineral-vitamin supplement. The close-up diet contained 465 g dry matter (DM)/kg fresh weight, 6.5 MJ/kg DM net energy of lactation (NEL), 142 g/kg DM crude protein, and 208 g/kg DM crude fibre. The early-lactation diet contained 450 g DM/kg fresh weight, 7.7 MJ/kg DM NEL, 178 g/kg DM crude protein, and 148 g/kg DM crude fibre.

Blood sampling and biochemical analysis

Ten blood samples were collected from each cow. First samples were planned to be obtained 14 days before the expected date of calving, but in fact they were collected 2–23 days before calving (week –1). Then they were collected in the peripartur period (1–4 days postpartum; week 0), next on day 7 (± 2 days) postpartum (week 1), and thereafter at weekly intervals until the end of the experiment (8 weeks postpartum). The sam-

ples were collected from the coccygeal vein into BD Vacutainer® rapid serum tubes (Becton Dickinson, Franklin Lakes, NJ, USA) at the same time on each sampling day (07:30–09:00). After centrifugation at $1\,600 \times g$ for 20 min, blood serum samples were separated and stored at -20°C until biochemical analysis. The biochemical analysis was performed at the laboratory of the Department of Veterinary Disciplines of the Czech University of Life Sciences in Prague, Czech Republic. Total protein (TP), albumin, urea, nonesterified fatty acids (NEFA), triglycerides (TG), cholesterol, and β -hydroxybutyrate (BHB) were measured using an Erba XL 200® automatic biochemical analyzer (Erba Mannheim, Mannheim, Germany) and commercial kits. NEFA and BHB were measured using Randox kits (Randox Laboratories, Crumlin, UK) while TP, albumin, urea, TG, and cholesterol were measured using Erba diagnostic kits (Erba Mannheim). Globulin was determined as the difference between total protein and albumin concentrations. In addition, the albumin-to-globulin ratio (A : G) was calculated.

Milk sampling and analysis

Eight milk composite samples (pooling milk from four quarters of the udder) were collected during afternoon milking at weekly intervals from week 1 to week 8 postpartum. The samples were collected on the same day as blood samples (± 1 day) into plastic tubes with a preservative containing a combination of Bronopol and Natamycin (Broad Spectrum Microtabs II®; D&F Control System, Inc., Dublin, CA, USA) and sent to the certified Laboratory for Milk Analysis in Buštěhrad, Czech Republic. The concentrations of milk fat, protein, and lactose were determined using a MilkoScan™ Fourier transform infrared spectroscopy (FTIR) analyzer (Foss Electric, Hillerød, Denmark).

Calculation and statistical analysis

All data (milk yield, blood and milk analyses results) were subjected to exploration analysis with the aim to identify outlying values and normality of distribution for all dependent variables. Data that were not normally distributed (NEFA, BHB, and cholesterol) were transformed to natural loga-

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rhythms. The cows selected for the experiment were blocked for parity into three groups (primiparous, parities 2 and 3, parity 4 and higher) and their numbers are given in Table 2.

Data were analyzed using a mixed linear model with repeated measures in the MIXED procedure of SAS (v9.3; SAS Institute Inc., Cary, NC, USA). Parameters were estimated by the restricted maximum likelihood method. The statistical model included the fixed effects of breed, week of lactation, parity within breed and their two-factor interactions, and the random effect of sampling day. Due to repeated measures within each cow, random covariances between weeks were summarized by residual R matrix. First-order autoregressive covariance structure with the random effect of animal was found to be the most appropriate in accordance with Akaike Information Criterion (AIC) and Schwarz Bayesian Criterion (SBC) (Littell et al. 2000). The least squares means (LSM) were calculated and multiple comparisons were made, with *P*-values adjusted using Tukey's procedure. The LSM of transformed variables and their confidence limits ($\alpha = 0.05$) were retransformed to the original scale.

RESULTS

The effects of cow breed on milk yield and composition during the first 8 weeks of lactation are shown in Figure 1. Except for week 1, H cows produced higher milk yield compared to F cows ($P < 0.05$). The percentage contents of protein were higher in F during the entire experimental period ($P < 0.05$), whereas the contents of fat were similar except for weeks 3 and 5, during which these were higher in F than in H cows (week 3: 5.8% vs 5.0%, respectively; week 5: 5.3% vs 4.6%, respectively; $P < 0.05$).

The development over time of energy profile blood parameters NEFA, BHB, TG, and cholesterol is shown in Figure 2. Blood NEFA concentrations prior to calving were low in both breeds, but they increased rapidly after calving in weeks 0 and 1 and then gradually decreased. Significantly ($P < 0.05$) higher concentrations were observed in H than in F (0.24 and 0.13 mmol/l, respectively) in week 4. Blood BHB concentrations were almost equal in the two breeds prior to calving. After calving in week 0, however, they were significantly higher ($P < 0.05$) in H compared to F (0.62 and

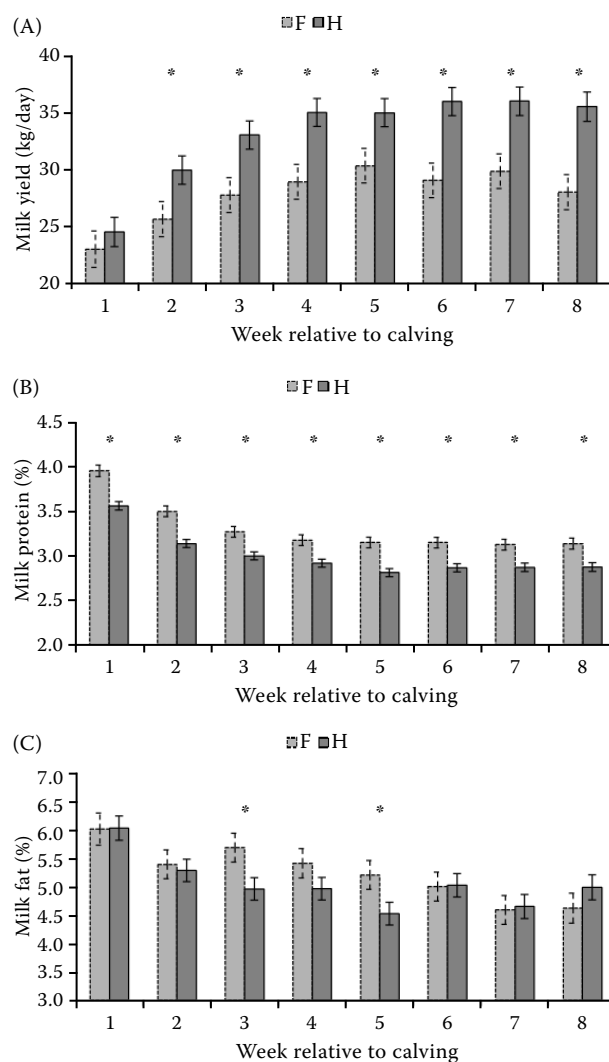


Figure 1. Means and standard errors of daily milk yield (A), milk fat¹ (B), and milk protein¹ (C) in Czech Fleckvieh (F) and Holstein (H) cows. Significant differences between breeds are indicated by * ($P < 0.05$)¹from afternoon milking

0.41 mmol/l, respectively), whereas no breed differences were observed over the remaining experimental period. The F cows had significantly higher TG concentrations before calving in week –1 compared to H cows (0.27 and 0.23 mmol/l, respectively; $P < 0.05$). These concentrations decreased and remained similar in the two breeds after calving. No breed differences were detected in cholesterol concentrations, which were lowest after calving and then steadily increased over the weeks.

Figure 3 shows the development of protein status blood parameters through the experimental period. TP concentrations were similar in both breeds before and early after calving. After week 1, numerically

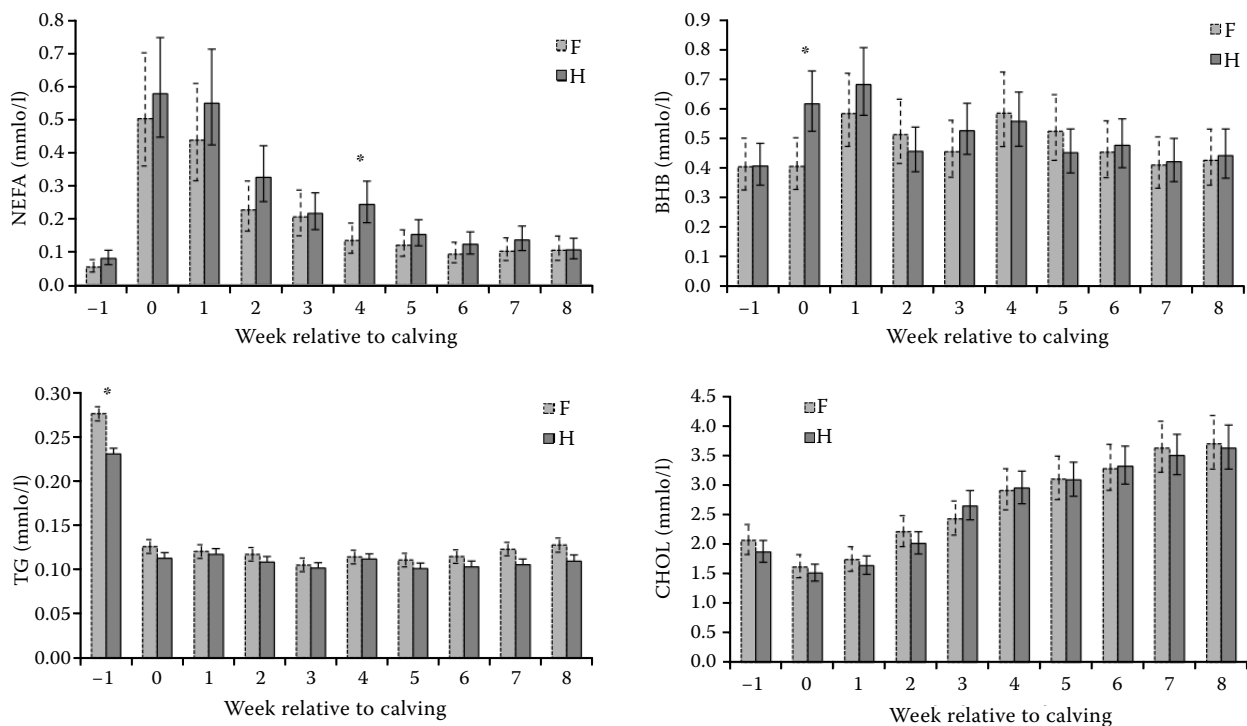
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Figure 2. Means and standard errors of triglycerides (TG) or 95% confidence interval limits of non-esterified fatty acids (NEFA), β -hydroxybutyrate (BHB), and cholesterol (CHOL) in Czech Fleckvieh (F) and Holstein (H) cows. Significant differences between breeds are indicated by * ($P < 0.05$)

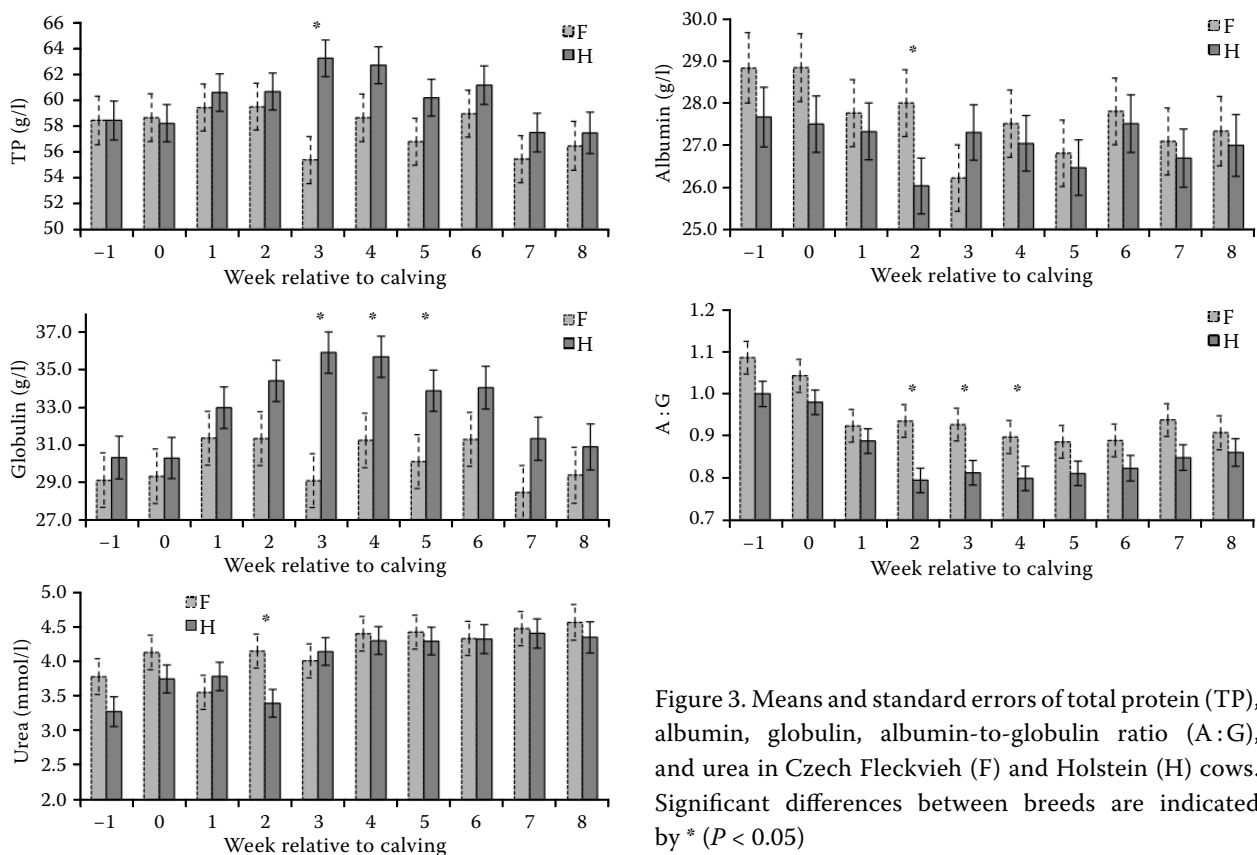


Figure 3. Means and standard errors of total protein (TP), albumin, globulin, albumin-to-globulin ratio (A:G), and urea in Czech Fleckvieh (F) and Holstein (H) cows. Significant differences between breeds are indicated by * ($P < 0.05$)

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higher TP concentrations were observed in H compared to F cows, with a significant difference seen in week 3 (63 and 55 g/l, respectively; $P < 0.05$). The breeds did not differ in albumin concentrations except that higher values ($P < 0.05$) were observed in F cows than in H cows (28 and 26 g/l, respectively) in week 2. Globulin concentrations were always numerically higher in H compared to F cows, with significant differences ($P < 0.05$) occurring in week 3 (36 and 29 g/l, respectively), week 4 (36 and 31 g/l, respectively), and week 5 (34 and 30 g/l, respectively). The A : G ratio was lower ($P < 0.05$) in H compared to F cows in week 2 (0.80 and 0.94, respectively), week 3 (0.81 and 0.93, respectively), and week 4 (0.80 and 0.90, respectively). Urea concentrations were significantly higher ($P < 0.05$) in F compared to H cows (4.2 and 3.4 mmol/l, respectively) only in week 2.

DISCUSSION

The present paper discusses the effects of two breeds differing in their production purpose (dairy vs dual-purpose breeds) on production characteristics and blood metabolic profile of cows in the periparturient period. Unlike as reported in most other studies investigating metabolic parameters in milk-producing cows, all animals used in this study were kept under identical management conditions.

Milk production

Milk yields observed in the present study over the experimental period were higher in H cows from week 2, whereas the contents of milk protein were always higher in F cows. These differences were expected and reflected different breeding objectives of the breeds (Barth et al. 2011) as well as average milk yields and protein contents achieved in the milk performance-recorded populations of F cows (7 297 kg and 3.55%, respectively) and H cows (9 740 kg and 3.36%, respectively) in the Czech Republic during 2017 (ICAR 2019). F cows produced milk with higher fat contents in weeks 3 and 5. Overall, the milk fat contents presented in this study (Figure 1) were higher than the averages reported for the country's overall populations of F and H cows (4.05% and 3.85%, respectively; ICAR

2019). That is probably due to the fact that the analyzed milk samples were collected during afternoon milking. As reported elsewhere, Swedish Red cows milked twice a day had 2% higher milk fat contents in afternoon milk compared with morning milk (Forsback et al. 2010).

Energy profile

In early lactation, dairy cows, especially of high-producing breeds, enter a state of NEB often associated with deterioration of health, production, and reproduction. The incidence of NEB may be indicated by increased serum NEFA concentrations, which reflect the mobilization of fat reserves (Danowski et al. 2012). This corresponds with the markedly increased NEFA values observed in the present study immediately after calving in both breeds. In this study, NEFA concentrations were higher in H cows during the entire experimental period. These differences were significant, however, only in week 4, even though the milk yields had differed significantly since week 2. The results obtained in a study investigating differences between German Red Pied and German Holstein breeds under the same management conditions were quite similar (Barth et al. 2011). German Holstein cows had markedly higher milk yields but the differences in serum NEFA were not significant. Other studies (e.g. Urdl et al. 2015) also reported only minor breed effects on NEFA concentrations, but it is important to emphasize that the cows of different breeds used in those experiments were managed differently. The cutoff point for NEFAs in detecting NEB and associated problems is 0.6 mmol/l (Van Saun 2016). In the present study, from week 0 to week 2 this threshold was exceeded at least once in 7 F (21%) and 21 H (50%) cows, when these animals may thus have entered a more serious state of NEB. The relatively higher frequency of exceeding the threshold for NEFA concentrations in H cows is in agreement with the higher average NEFA concentrations recorded in this breed throughout the experiment (albeit mostly without statistical significance).

NEFA transformation to ketones, including BHB, is a common metabolic pathway to provide energy to dairy cows during early lactation. The synthesis of large amounts of ketones, however, may result in ketosis (Duffield et al. 2009). Our data indicate a marked increase of BHB concentrations

in H cows just around calving (week 0), whereas F cows exhibited similar BHB values in week 0 like in week –1. The BHB levels increased in week 2 also in F cows, and therefore the breed difference was less pronounced. Nevertheless, in the first 2 weeks after calving, only 1 F cow (4%) compared to 11 H cows (46%) had BHB values falling into the interval indicating subclinical ketosis (from 1.2 mmol/l to 2.9 mmol/l; [McArt et al. 2012](#)), and 1 H cow reached the cutoff point for clinical ketosis (> 2.9 mmol/l; [McArt et al. 2012](#)). Therefore it is obvious that the number of cows with increased risk of NEB and subclinical ketosis indicated greater metabolic problems in the H breed. Higher BHB concentrations during the transition period were previously observed also in dairy breeds Brown Swiss and Holstein compared to the dual-purpose Simmental breed ([Urđl et al. 2015](#)). A twice-a-day milking system was used in our study. Increasing the daily milking frequency might be associated with a higher metabolic imbalance in early lactation. Indeed, cows milked three times daily had a 19% higher plasma concentration of BHB compared with cows milked twice daily ([Andersen et al. 2004](#)).

TG concentrations constituted the only parameter affected by breed at the prepartum sampling in this study, with lower values detected in H cows. TG level decreased from week –1 to week 0 in both breeds, indicating a considerable change in the energy balance status of cows in the peripartum period. This may have been due to TG uptake by the mammary gland for milk fat synthesis, increased lipolysis and decreased lipogenesis, or limited liver capability to complete lipoproteins as TG's transportable form ([McArt et al. 2013](#)). Reference values for serum TGs range from 0.14 mmol/l to 0.27 mmol/l ([Cozzi et al. 2011](#)). Thus, the average TG values in lactating cows of both breeds were below the normal threshold during our experiment. This may indicate energy deficit and the development of liver steatosis characterized by TG accumulation in the liver ([Drackley et al. 2001](#)).

Cholesterol is a component of serum lipoproteins, and its concentration in serum is an indication of overall lipoprotein concentrations. Reference values for serum cholesterol range from 1.11 mmol/l to 8.57 mmol/l in dairy cows ([Oregon State University 2019](#)), whereas a range from 1.68 mmol/l to 2.95 mmol/l has been determined for close-up dry cows ([Pennsylvania State](#)

[University 2013](#)). These limits of normal values were not exceeded in this experiment at any of the sampling times. The moderate decrease in serum cholesterol during the last stage of pregnancy is likely due to the increased requirement of foetal tissues as well as maternal glands for steroid hormone synthesis ([Pysera and Opalka 2000](#)). The cholesterol concentrations are lowest in early lactation ([Jozwik et al. 2012](#)). The cholesterol level increased over time after calving, which is a physiological effect necessary to ensure sufficient VLDL synthesis to avoid accumulation of TGs in the liver ([Gross et al. 2013](#)). No difference in serum cholesterol was found between the breeds in this study, and this may indicate the same degree of lipomobilization and need of cholesterol for VLDL hepatic synthesis. In contrast, [Urđl et al. \(2015\)](#) reported lower postpartum cholesterol levels in dual-purpose Simmental cows compared to dairy Holstein and Brown Swiss cows throughout the entire 105 days of that study's observation period.

The dynamics of the energy status indicators shows that the cows of both breeds underwent NEB especially in the first 2 weeks after calving. The onset of changes was rather faster and more pronounced in the H breed. Nevertheless, it seems that the induced level of metabolic stress related to liver functions was similar in the cows of both breeds.

Protein status

The concentration of serum proteins is influenced by environmental factors (nutritional management, season, climate, etc.), breed, and such individual characteristics as stage of lactation, parity, and health condition ([Alberghina et al. 2011](#)). The reported reference values range from 58 g/l to 80 g/l for serum total protein, from 22 g/l to 36 g/l for serum albumin, from 24 g/l to 40 g/l for total serum globulin ([Kessel 2015](#)), from 0.42 to 1.34 for the A : G ratio ([Alberghina et al. 2011](#)), and from 2.9 mmol/l to 9.6 mmol/l for blood urea ([Oregon State University 2019](#)). In this study, serum TP concentrations were at the lower limit whereas the other parameters of protein status fell within the physiological limits mentioned above.

Changes in concentrations of albumins and of globulins and in the A : G ratio were reflected against a background of higher postpartum TP

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concentrations in H cows (significant difference only in week 3). Breed differences in the dynamics of albumin concentration development are apparent particularly in weeks 2 and 3. Whereas H cows reached the lowest levels of albumin concentrations during week 2, this occurred in F cows during week 3. Globulin concentrations developed differently in the two breeds. Whereas these remained quite similar in F cows throughout the experiment, H cows exhibited increased values until week 3, then a gradual decrease, with the differences in weeks 3, 4, and 5 being significant. The significantly higher A : G ratios in F cows during weeks 2, 3, and 4 are mainly due to increased globulin concentrations in H cows.

As with our results, H cows had higher concentrations of TP and total globulin and lower albumin concentrations and A : G ratio in comparison with dual-purpose breeds in the study by Bobbo et al. (2017). The authors hypothesized that this might have been due to greater selection pressure for milk yield, although no effect of milk performance alone on TP values was demonstrated and higher albumin levels were observed in high milk-yielding herds (> 30 kg/day). Those authors also considered the effect of more intensive nutrition provided in herds with higher milk yields, as positive effects of high-protein diet on TP and albumin levels had been repeatedly reported earlier (Raggio et al. 2007).

Slightly higher TP concentrations in H cows and lower concentrations of albumins in F cows shown in this study may indicate poorer nutrient supply associated with the NEB at the onset of lactation. The observed changes, then, may reflect a greater metabolic stress and particularly deteriorated liver functions in H cows, as already indicated by energy profile parameters. Albumin and most globulins are synthesized in hepatocytes, except that a gamma fraction of globulins is produced in plasma cells and constitutes approximately 12–20% of all globulins. This fraction serves as an indicator of immune response (Tothova et al. 2016). Impaired liver functions, such as in the case of fatty liver syndrome, result in reduced synthesis of albumin (Sevinc et al. 2001), whereas TP levels may remain unchanged due to the increase of gamma globulins associated with subsequent immune response.

The serum urea values measured in this study fall within the physiological limits for dairy cattle ranging from 2.9 mmol/l to 9.6 mmol/l (Oregon State University 2011). Urea levels varied over the first

four sampling times in the present experiment. Subsequently, they stabilized at values 15–30% higher than those prior to calving. While these relatively stable values corroborate the intensive intake and metabolism of nitrogen substances over a long period, the interpretation of urea dynamics during the first weeks of monitoring is more difficult. On the one hand, more intensive nutrition and metabolism pushed urea levels up. On the other hand, the milk production led to a relative deficit. The breed differences corresponded to the dynamics of albumin, with a tendency toward higher values in F cows during week –1 ($P = 0.07$) and a significant dominance of F cows in week 2. Whereas the tendency at the beginning of the experiment probably reflects better fulfilment of F cows' requirements, the differences in week 2 might be interpreted as a protein deficit in H cows accompanied by maximal utilization of nitrogen substances. Our findings correspond to the results published by Urdl et al. (2015), who found lower urea values in Holstein compared to Simmental cows and at a low-energy diet in contrast to a diet matching nutrition standards.

CONCLUSION

The comparison of milk production and selected parameters of metabolic profile between dairy H and dual-purpose F cows kept under identical management conditions showed that, despite the H breed's higher milk yields, the levels of metabolic stress assessed according to blood concentrations of NEFA, BHB, TG, and cholesterol, as well as of TP, albumin, and urea were similar in both breeds. Postpartum concentrations of total globulins were higher and the A : G ratio was lower in H compared to F cows. The dynamics of the energy status indicators shows that the cows of both breeds underwent NEB accompanied by some protein metabolism changes, and especially in the first 2 weeks after calving. It seems that the induced level of metabolic stress related to liver functions was similar in the cows of both breeds. Certain impairment of liver functions was indicated by low levels of albumins in both breeds and increased levels of globulins in H cows due to a stronger immune response. It seems that the onset of changes was rather faster and more pronounced in the H breed. Further research is needed to verify whether the re-

sults obtained are breed-specific depending on the animals' production purpose or whether they simply reflect different milk yields. The results can be useful for herd management purposes in relation to the timings of various preventive measures. Determining the direct indicators of energy balance used in this study is expensive and difficult. Therefore, more attention should be given to their relationships with indirect indicators that are more easily applicable under farm conditions and whose potential is currently increasing due to rapid technological progress.

Conflict of interest

The authors declare no conflict of interest.

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