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Kidney function, but not nitrogen excretion differs between Brown Swiss and Holstein dairy cows

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ABSTRACT

Brown Swiss (BS) cows have greater urea concentrations in milk and blood compared with Holstein (HO) cows. We tested the hypothesis that BS and HO cows differ in kidney function and nitrogen excretion. Blood, saliva, urine, and feces were sampled in 31 multiparous BS and 46 HO cows kept under identical feeding and management conditions. Samples were collected at different lactational stages after the monthly DHIA control test-day. To test the glomerular filtration rate (GFR) and urea excretion, concentrations of creatinine and urea were measured in serum, urine, and saliva. As an additional marker to estimate GFR, we determined symmetric dimethylarginine (SDMA) in serum. Feces were analyzed for dry matter content and nitrogen concentration. Data on milk urea and protein concentrations, and daily milk yield were obtained from the monthly DHIA test-day records. The effects of breed, time, and parity number on blood, saliva, urine, feces, and milk parameters were evaluated with the GLM procedure with breed, time, and parity number as fixed effects. Differences between BS and HO were assessed by the Tukey-corrected *t*-test at $P < 0.05$. Concentrations of urea, creatinine, and SDMA in serum, were greater in BS than in HO cows ($P < 0.01$): 5.46 ± 0.19 vs 4.72 ± 0.13 mmol/L (urea), 105.96 ± 2.23 vs 93.07 ± 1.50 mmol/l (creatinine), and 16.78 ± 0.69 vs 13.39 ± 0.44 µg/dL (SDMA). We observed a greater urea concentration in BS cows (25.8 ± 0.7 vs 21.8 ± 0.7 mg/dL) and protein content in milk (3.70 ± 0.08 vs 3.45 ± 0.07%) than in HO cows ($P < 0.01$). Urea and creatinine concentrations in urine and saliva did not differ among breeds. No differences between BS and HO were observed for milk yield, fecal DM, and fecal nitrogen content. Dry matter intake and body weight were similar in BS and HO cows ($P > 0.05$). Despite greater urea, creatinine, and SDMA concentrations in blood as well as a higher milk urea content in BS compared with HO, respective concentrations in urine did not differ between breeds. In conclusion, our results demonstrate a lower renal GFR in BS compared with HO cows, thereby contributing to the greater plasma urea concentration in BS cows. However, estimation of nitrogen excretion via milk, urine, and feces does not entirely reflect nitrogen turnover within the animal.

Key words: urea, creatinine, nitrogen excretion, Holstein, Brown Swiss, dairy cow

INTRODUCTION

The microbial degradation of dietary proteins in the rumen is catalyzed by proteolytic enzymes and leads to the production of ammonia. Ruminal microorganisms use ammonia nitrogen for their amino acid and protein synthesis. Excessive ammonia is transported across the rumen wall and reaches the liver via the portal vein for detoxification (Kristensen et al., 2010). Hepatocytes convert rumen-derived ammonia and nitrogenous compounds from the intermediary metabolism to urea and release it into circulation for excretion via the kidneys (Røjen et al., 2011). Besides, urea diffuses or is transported into several other body fluids and secretions, as for instance milk and saliva, and arrives in the rumen and the gut serving as nitrogen source for microbial protein synthesis (Lapierre and Lobley, 2001). Thus, ruminants can recycle part of the urea via the so-called ruminohepatic cycle.

Blood urea represents the main end product of nitrogen metabolism before excretion in ruminants. Excretion of abundant nitrogen occurs mainly via urine (33%), feces (35%) and milk (26%; Spek et al., 2013). Because milk urea concentration closely reflects blood urea concentration (Broderick and Clayton, 1997; Burgos et al., 2007; Kessler et al., 2020), it is often used as a tool to evaluate nutrition in terms of crude protein and energy supply for rumen fermentation in dairy cows (Hof et al., 1997; Nousiainen et al., 2004; Bastin et al., 2009). Due to the significant relationship between milk urea nitrogen and urinary urea nitrogen, milk urea is further used to predict nitrogen excretion via urine (Jonker et al., 1998; Kauff-

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man and St-Pierre, 2001; Nousiainen et al., 2004). However, the equations used for nitrogen balance calculations at farm level base upon data collected almost exclusively in Holstein cows (Jonker et al., 1998; Kohn et al., 2002; Broderick, 2003), and potential differences among dairy cow breeds are not considered.

It is well known that milk urea content is affected by the crude protein level in the diet, yet milk urea also differs between breeds. Holstein (HO) cows had lower concentrations of urea in milk compared with Ayrshire (Miglior et al., 2006) and Brown Swiss (BS) cows in studies based on the evaluation of test-day records (Wattiaux et al., 2005; Doska et al., 2012; Kessler et al., 2020). Since data about the feeding regimen were scarcely provided in these studies, a dietary effect contributing to the differences in milk urea between BS and HO cows must be considered. Recently, greater blood and milk urea concentrations in BS compared with HO cows were confirmed even when cows of both breeds were kept under identical feeding and management conditions (Kessler et al., 2020). Based on breed differences in blood and milk urea content, also the overall nitrogen excretion can be assumed to differ between BS and HO. However, it is still unknown if greater blood urea concentrations are congruently reflected by concomitantly elevated contents of nitrogenous compounds in other media (e.g., urine and saliva). On the other hand, we can speculate if less N-excretion in one compartment is compensated by greater concentrations in other media. Based on earlier observations showing breed differences in urea contents of milk and blood (Murayama et al., 2014; Kessler et al., 2020), a differential kidney function could explain differences between BS and HO cows.

The objective of the present study was to simultaneously assess biomarkers for kidney function like urea, creatinine, and symmetric dimethylarginine (SDMA) concentrations in several body fluids (i.e., blood, urine, milk, and saliva) as well as nitrogen excretion in feces in BS and HO cows kept under identical feeding and management conditions. We aimed at investigating differences in renal function and nitrogen excretion between BS and HO dairy cows that could explain phenotypic breed differences.

MATERIALS AND METHODS

Animals, Feeding and Sampling

The present experiment was performed in line with the Swiss law of animal protection and was approved by the Committee of Animal Experiments of the Canton Fribourg, Switzerland (2018_19_FR). This study included 31 lactating Brown Swiss (BS, parity number 3.1 ± 0.3; range 1 – 8; parity number 1: n = 4; parity number 2 + 3: n = 16; parity number >4: n = 11) and 46 Holstein (HO, parity number 2.2 ± 0.2, mean ± SEM; range 1 – 5; parity number 1: n = 17; parity number 2 + 3: n = 22; parity number >4: n = 7) dairy cows that were kept on one commercial dairy farm in the Swiss Midlands under identical feeding and management conditions. Previous lactation yields were 8,357 ± 185 kg for BS and 8,933 ± 272 kg for HO (mean ± SEM). All cows were co-housed as one herd in the same free-stall barn, milked twice daily in a milking parlor and fed 3 × /d a partial mixed ration (PMR) based on corn and grass silage, wet brewers grains, as well as soybean and rapeseed meal. Additionally, all cows (BS and HO) received 2 kg/d of a 18% concentrate mix in transponder feeding stations (UFA 164 F EXTRA, UFA AG, Switzerland) during the first 4 mo of lactation. The composition and nutrient values of the PMR and concentrate are shown in Table 1.

Blood, saliva, and spot urine samples were collected in parallel between 0700 h and 0900 h on the morning following the monthly DHIA test-day at 2 occasions. Blood (BS: n = 31, HO: n = 46) was sampled from the coccygeal vein with evacuated tubes containing a clot activator (Vacuette, Greiner Bio One, Kremsmünster, Austria) and was allowed to clot for 30 min at room temperature before centrifugation at 2,500 × g (20 min, 4°C) to obtain serum. Saliva samples (BS: n = 29, HO: n = 44) were obtained using Salivette (Sarstedt, Nuembrecht, Germany) saliva collection tubes, whose sponges were introduced in the oral cavity for approximately 1.5 min until being soaked with saliva. Saliva samples were immediately stored on wet ice until centrifugation (2,500 × g for 20 min at 4°C) to harvest saliva. For the collection of spot urine samples (50 mL; BS: n = 16, HO: n = 24), cows were induced to urinate by mild vulvar stimulation

Table 1. Composition and nutrient values of the partial mixed ration (PMR; n = 5) and concentrate fed to all cows (mean ± SD)

Item	PMR	Concentrate[1]
Components (% in fresh matter)		
Corn silage	59.5	
Grass silage	23.8	
Wet brewers grain	7.9	
Alfalfa hay	3.9	
Soybean and rapeseed meal	3.0	
Straw, minerals	1.9	
Nutrient values		
DM content (%)	413.7 ± 27.9	880
Energy (MJ NEL/kg DM)	6.5 ± 0.1	7.6
Crude fiber (g/kg DM)	ND	35
Crude ash (g/kg DM)	63.5 ± 4.2	45
Crude fat (g/kg DM)	33.2 ± 1.4	55
CP (g/kg DM)	135.4 ± 4.7	180
ADF (g/kg DM)	209.7 ± 8.9	ND
NDF (g/kg DM)	373.4 ± 15.0	ND
Lignin (g/kg DM)	22.8 ± 1.8	ND

[1]UFA 164 F EXTRA (UFA AG, Switzerland) fed to all cows during the first 4 mo of lactation at 2 kg/d in transponder feeding stations.

lasting for maximum 10 min. Urine spot samples were obtained and cooled on wet ice until transportation to the lab. Aliquots of serum, saliva, and urine were stored at −20°C until analysis. After blood and saliva sampling, fecal samples (BS: n = 31, HO: n = 43) were taken manually from the rectum. About 50g of feces were transferred to plastic containers, put on wet ice for transportation to the lab and stored frozen at −20°C until analysis.

Estimation of Body Weight and Feed Intake

Heart girth was assessed with a plastic-coated measuring tape as the minimal circumference around the body and being placed directly behind the elbows. The measuring tape was validated for estimating BW from heart girth in HO and BS cows (Gruber et al., 2018). Based on breed, parity number, lactational stage, estimated body weight, milk yield, energy density and composition of the diet, DMI of cows was estimated using a prediction model proposed by Gruber that was evaluated for its accuracy by Jensen et al. (2015).

Laboratory Analysis

Feed Sample Analysis. Chemical composition and nutrient values of the PMR feed samples (n = 5) were assessed according to accredited AOAC methods (e.g., determination of crude ash, crude fat, N content (Dumas method), ADF, NDF, and lignin) described by Heublein et al. (2017). The PMR energy content was calculated according to the German Society of Nutrition Physiology (GfE, 2004). Chemical composition of the used batch of concentrate was provided by the manufacturer UFA AG, Switzerland.

Milk Analysis. Data on milk urea and protein concentrations, and daily milk yield were obtained from the monthly DHIA test-day records. Analysis of milk urea and protein content was performed with an infrared milk analyzer (MilkoScan FT, Foss Analytical A/S, Hillerød, Denmark) by Suisselab AG (Zollikofen, Switzerland).

Analysis of Urine, Blood, and Saliva. Urea and creatinine concentrations in serum, saliva, and urine were measured with commercially available kits from Axon Lab (#10221D, Baden, Switzerland) and DiaSys Diagnostic Systems (Creatinine FS 1 1711 99 10 021, Holzheim, Germany), respectively. Activity of creatine kinase in serum was determined with a commercially available kit (AXON00005, Axon Lab, Baden, Switzerland). Symmetric dimethylarginine concentration in blood was determined with a SDMA Slide from IDEXX Laboratories, Inc. (One IDEXX Drive, Westbrook, Maine 04092, United States) run on a Idexx Catalyst One Chemistry Analzyer. Free fatty acids and BHB concentrations in serum were assessed using the commercially available kits #FA 115 and RB 1007, respectively (both from Randox Laboratories Ltd., Schwyz, Switzerland). Insulin-like growth factor-1 (IGF-1) in serum was assessed by RIA as described earlier (Vicari et al., 2008). Intra- and inter-assay CVs of IGF-1 were < 10%.

To assess DM content of saliva and urine, 1 mL of saliva and 5 mL of urine were dried in a glass vial during 4 h at 105°C. Saliva and urine samples were further measured with an optical Brix refractometer (Manual Refractometer MHRB-40 ATC, Mueller Optronic, Erfurt, Germany) with a scale ranging from 0 to 40% Brix. The refractometer was equipped with an automatic temperature compensation mechanism to warrant accurate measurements without recalibration after shifts in ambient working temperature. According to the manufacturer, the accuracy of the instrument was ± 0.2% Brix at 20°C. Osmolality in urine was measured with an osmometer (K-7400S, Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany).

Analysis of feces. Dry matter content in fecal samples was determined by oven-drying at 60°C for 48 h. Nitrogen content in feces was analyzed by the Kjeldahl method as described in VDLUFA (III, 2012; method 4.1.1).

Statistical Analysis.

The statistical software package SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. Data presented in this study are mean values ± SEM. Normal distribution of data was checked with the UNIVARIATE procedure of SAS. The effects of breed (HO, BS), time (date of sampling relative to parturition), and parity number (1 to 8) on parameters measured in blood, saliva, urine, milk, and feces as well as DMI and BW were evaluated with the GLM procedure with time and parity number as fixed effects. Significance of breed, time and parity number on study variables was estimated with the Tukey-corrected *t*-test. Significant effects were considered at $P < 0.05$. Correlations of salivary and urinary DM content with the corresponding Brix values were calculated using the REG procedure.

RESULTS

Performance and Metabolic Status

Milk yield and protein content increased with DIM ($P < 0.0001$, Figures 1A and 1B). Brown Swiss cows showed higher protein concentrations in milk compared with Holstein cows ($P < 0.001$), while milk yield did not differ between BS and HO cows. Multiparous cows produced more milk than primiparous cows ($P < 0.01$). Body weight changed with lactational stage but was not

Table 2. Blood metabolites, IGF-1 concentration, and body weight (BW) in Brown Swiss (BS: n = 31) and Holstein (HO: n = 46) cows (mean ± SEM). Effects of breed (BS vs. HO), time (date of sampling relative to parturition), and parity number on BW, blood metabolites, and IGF-1 concentration were considered significant at $P < 0.05$

	Breed		*P*-values		
Parameter	BS	HO	Breed	Time	Parity
BW (kg)	674 ± 9	693 ± 7	$P = 0.10$	$P < 0.0001$	$P = 0.48$
FFA (mmol/L)	0.06 ± 0.01	0.10 ± 0.02	$P = 0.09$	$P < 0.01$	$P = 0.14$
BHB (mmol/L)	0.65 ± 0.05	0.68 ± 0.04	$P = 0.63$	$P = 0.15$	$P = 0.91$
IGF-1 (ng/mL)	121 ± 10	138 ± 6	$P = 0.50$	$P = 0.01$	$P < 0.05$
Creatine kinase (U/L)	162.5 ± 12.1	158.9 ± 15.2	$P = 0.86$	$P = 0.16$	$P = 0.37$

affected by parity number or breed (Table 2). Dry matter intake did not differ between breeds (data not shown).

Concentrations of FFA and BHB in serum did not differ between breeds ($P = 0.09$ and $P = 0.63$, respectively; Table 2). Serum concentrations of IGF-1 were not related with breed ($P = 0.50$; Table 2). Creatine kinase concentration in serum showed no changes over time and was neither affected by breed nor by parity (Table 2).

Parameters Related to Kidney Function, Nitrogen Turnover and Excretion

Urea concentrations in serum, urine, milk, and saliva did not change with DIM (Figures 2A, 2B, 2C, and 2D). As expected, concentrations of urea in serum and milk were higher in BS compared with HO cows ($P < 0.01$), whereas urea concentrations in urine and saliva did not differ between breeds. Parity did not affect urea concentrations in serum, urine, milk, and saliva. Creatinine concentrations in serum and urine increased with DIM ($P < 0.0001$ and $P < 0.01$, respectively), while saliva urea concentrations remained on a similar level throughout lactation (Figures 3A, 3B, and 3C). Figure 4 shows the relationship between blood urea nitrogen and milk urea nitrogen content in BS and HO cows. Serum creatinine concentration was greater in BS than in HO ($P < 0.0001$), in contrast to creatinine concentration in urine and saliva where no differences between breeds were detected. Serum and urine creatinine concentrations were affected by parity number, but no consistent progression could be defined ($P < 0.05$). However, parity was not associated with saliva creatinine concentration. Concentration of SDMA in blood was neither affected by DIM, nor by parity (Figure 5). SDMA in serum was higher in BS compared with HO ($P < 0.001$).

Dry matter content and Brix values in urine and saliva were not affected by DIM (Figures 6A, 6B, 6C, and 6D). Breed and parity were not associated with DM and % Brix in urine and saliva. In urine and saliva, DM and % Brix showed a clear correlation (r = 0.94 and r = 0.95, respectively, Figures 6E and 6F). Osmolality in urine did not differ between BS and HO (data not shown). Fecal DM content and nitrogen content were neither affected by breed, nor time or parity (Table 3).

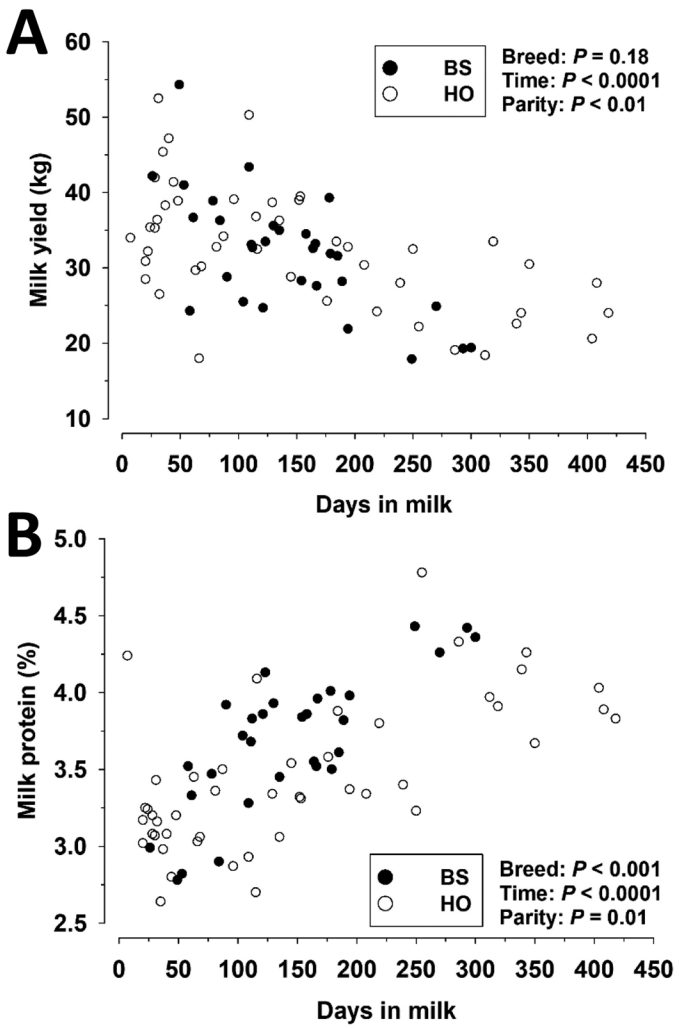


Figure 1. Milk yield (A) and milk protein content in % (B) in Brown Swiss (BS: n = 31) and Holstein (HO: n = 46) cows. Effects of breed (BS vs. HO), time (date of sampling relative to parturition), and parity number on milk yield and milk protein content were considered significant at $P < 0.05$.

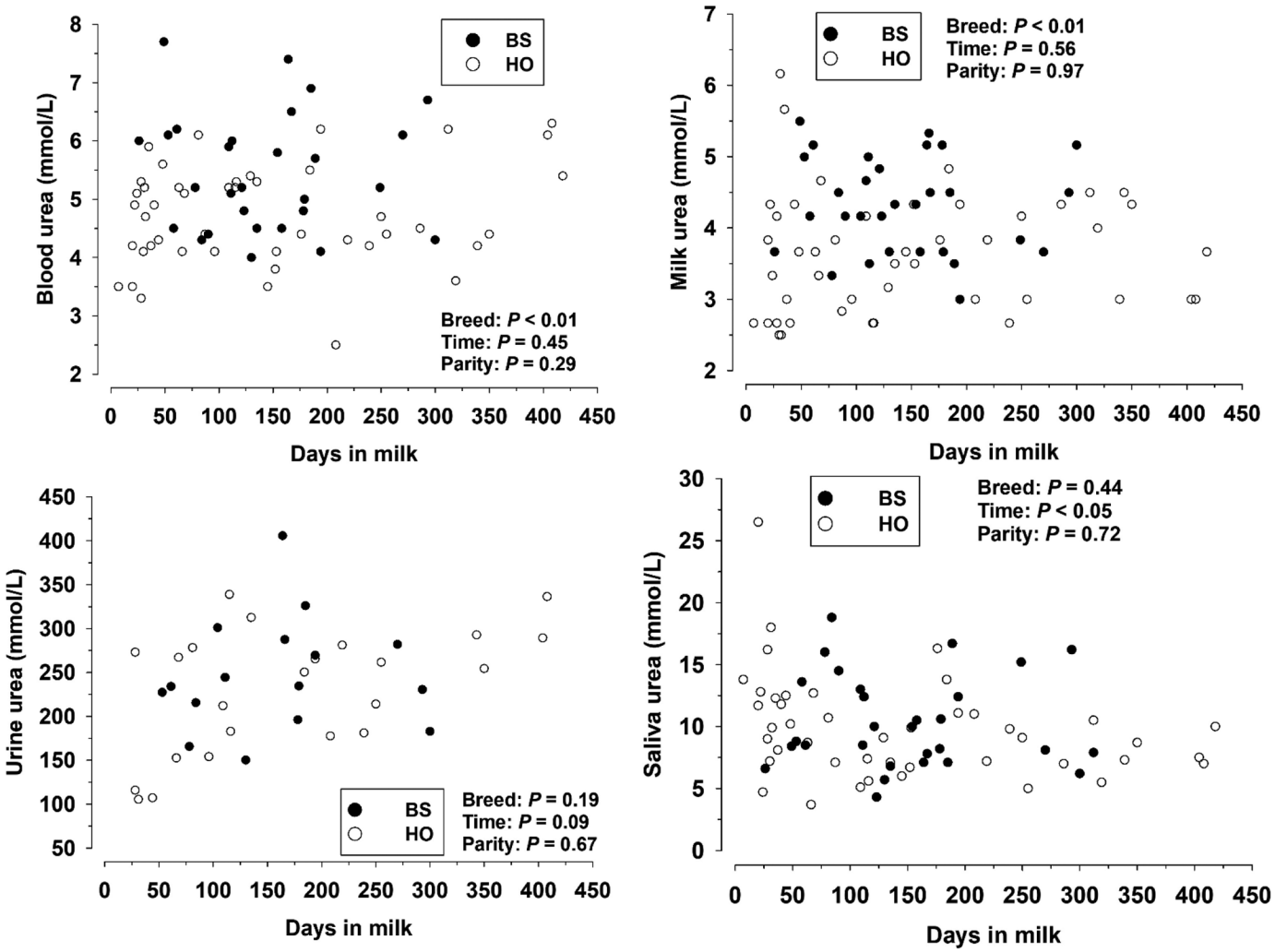
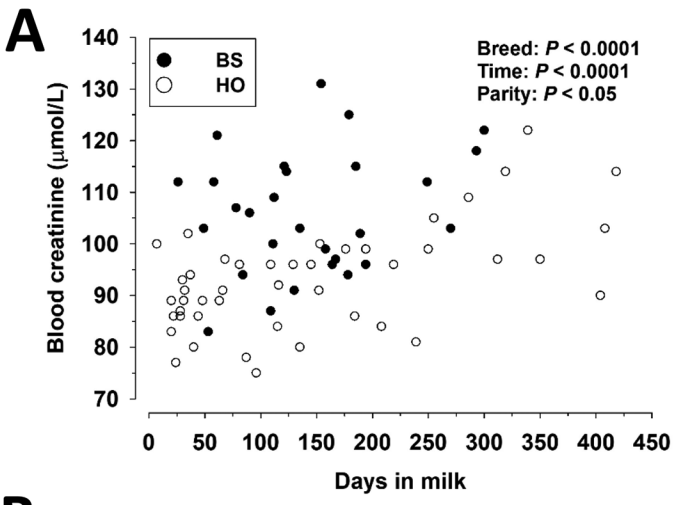


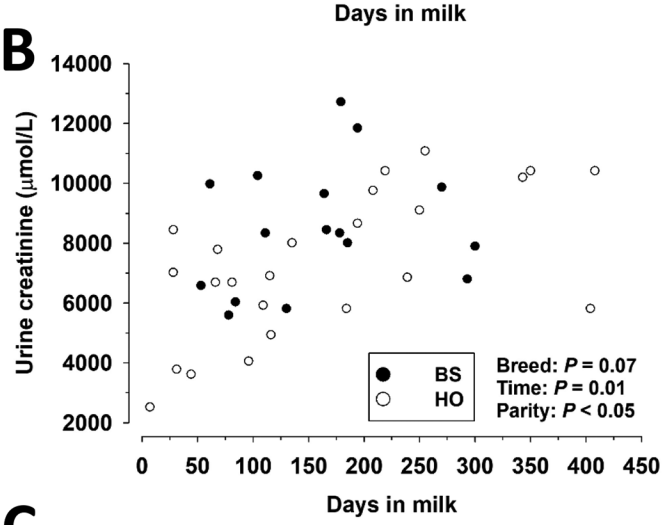
Figure 2. Urea concentrations in blood (A; BS: n = 31, HO: n = 46), urine (B; BS: n = 16, HO: n = 24), milk (C; BS: n = 31, HO: n = 46), and saliva (D; BS: n = 29, HO: n = 44) in Brown Swiss (BS) and Holstein (HO) cows. Effects of breed (BS vs. HO), time (date of sampling relative to parturition), and parity number on urea concentration in blood, urine, milk, and saliva were considered significant at $P < 0.05$.

DISCUSSION

Higher milk urea concentrations in Brown Swiss compared with Holstein Friesian cows were reported in cows fed different diets (Wattiaux et al., 2005; Doska et al., 2012; Kessler et al., 2020) as well as in animals kept under identical feeding conditions (Kessler et al., 2020). Hence, the question arises if BS cows also have greater urea concentrations in body fluids other than blood and milk. Due to its essential role in nitrogen turnover, it is obvious that the kidney could contribute to the phenotypic differences observed between cow breeds. The present study therefore investigated indicators of renal function like urea, creatinine, and SDMA in blood and urine as well as markers of urea and nitrogen turnover by simultaneously measuring urea and nitrogen concentrations in different body fluids (i.e., milk, urine, saliva, and feces) in BS and HF cows.

Cows enrolled in the present experiment were kept under identical feeding and management conditions. Since milk production, BW, and DMI was similar among BS and HO cows, differences in nitrogen and urea metabolism are likely to originate from physiological differences in nitrogen metabolism and turnover. In addition, cows of both breeds showed a similar range in serum BHB and IGF-1concentrations indicating a comparable metabolic status in BS and HO cows. The slightly greater serum FFA concentrations in HO cows were likely due to a couple of early lactating HO cows still exhibiting high FFA concentrations. Based on our measurements on serum creatine kinase concentrations, muscle damage or severe muscle depletion can be excluded.





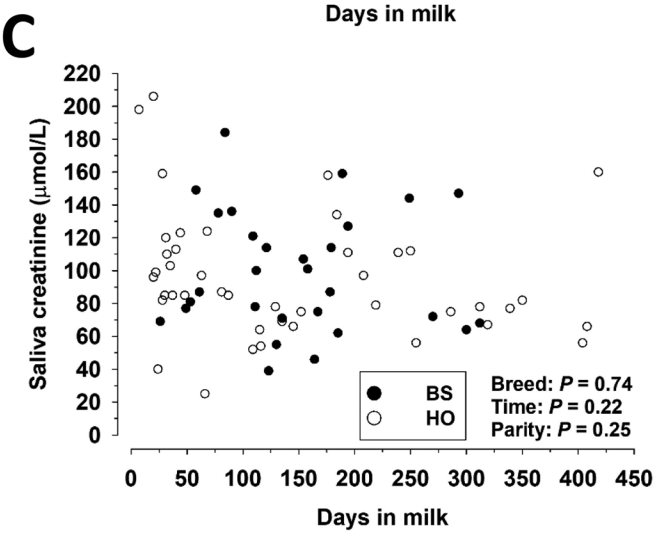


Figure 3. Creatinine concentrations in blood (A; BS: n = 31, HO: n = 46), urine (B; BS: n = 16, HO: n = 24), and saliva (C; BS: n = 29, HO: n = 44) in Brown Swiss (BS) and Holstein (HO) cows. Effects of breed (BS vs. HO), time (date of sampling relative to parturition), and parity number on creatinine concentration in blood, urine, and saliva were considered significant at $P < 0.05$.

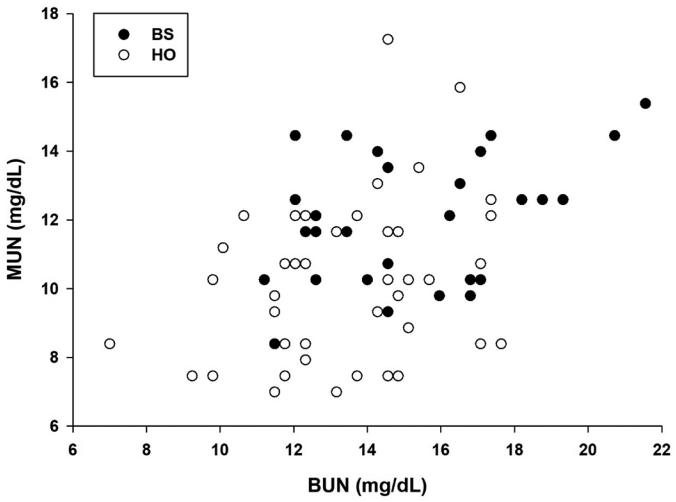


Figure 4. Relationship between blood urea nitrogen (BUN) and milk urea nitrogen (MUN) content in Brown Swiss (BS, n = 31) and Holstein (HO, n = 46) cows.

As expected, we observed higher urea concentrations in blood and milk in BS compared with HO cows. One might therefore assume that BS also have greater urea concentrations in urine and saliva. However, we did neither observe differences in the concentrations of urea in urine nor in saliva between breeds. At first sight, these findings might be related to differences between breeds in the endogenous urea transport. In ruminants, urea uptake in the gastrointestinal tract (especially rumen) provides nitrogen for microbial protein synthesis

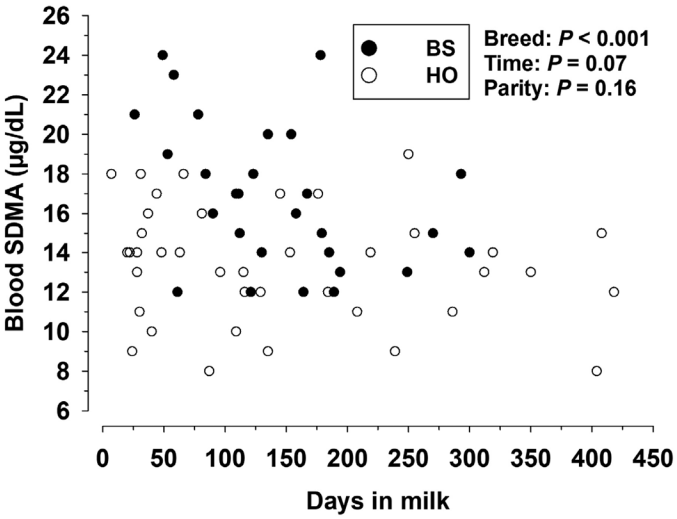


Figure 5. Symmetric dimethylarginine (SDMA) concentration in blood in Brown Swiss (BS: n = 31) and Holstein (HO: n = 46) cows. Effects of breed (BS vs. HO), time (date of sampling relative to parturition), and parity number on SDMA concentration in blood were considered significant at $P < 0.05$.

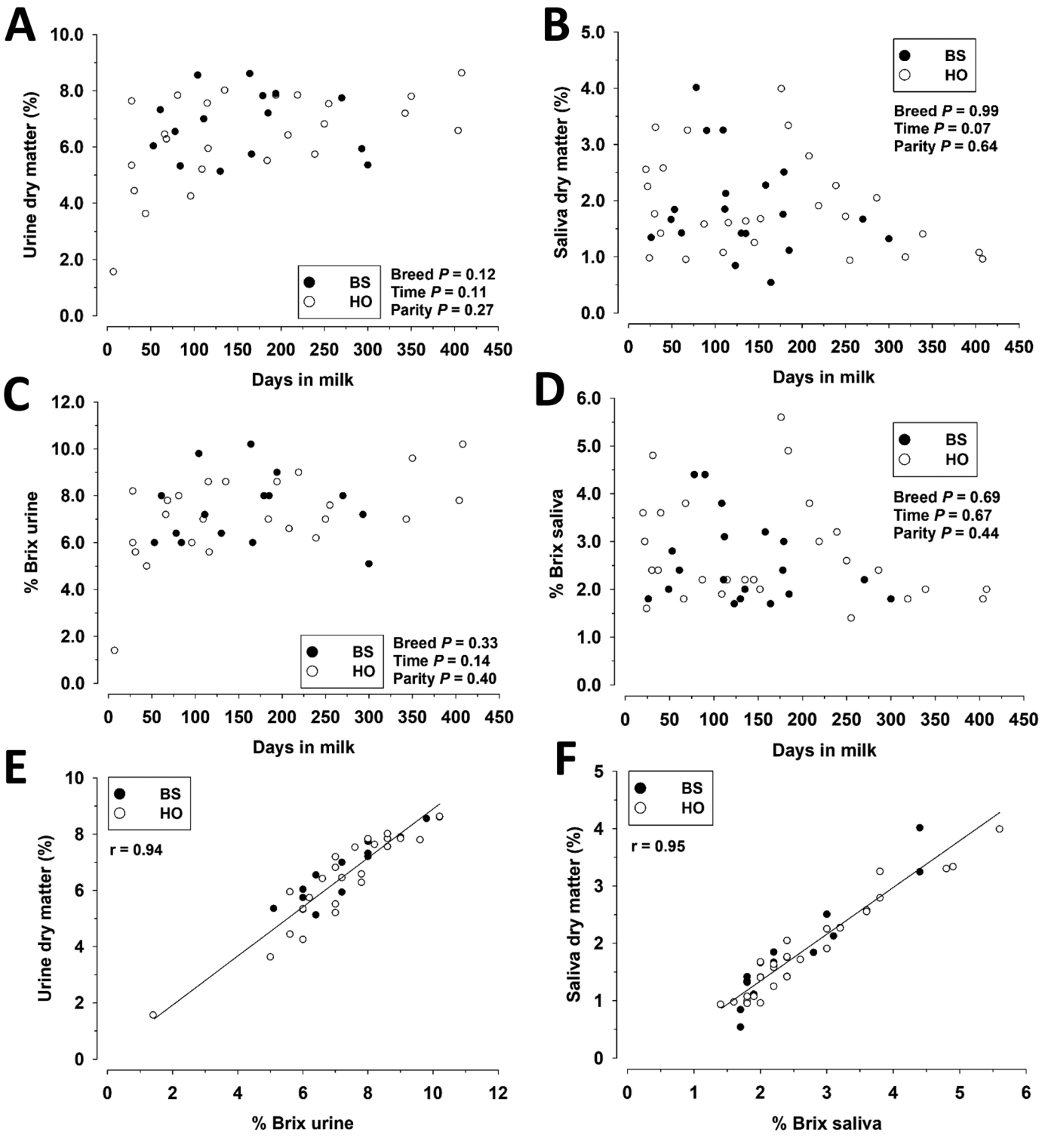


Figure 6. Dry matter (DM) content in urine (A) and saliva (B), % Brix in urine (C) and saliva (D), and DM content in relation to % Brix in urine (E) and saliva (F) in Brown Swiss (BS: urine n = 16, saliva n = 29) and Holstein (HO: urine n = 24, saliva n = 44) cows. Effects of breed (BS vs. HO), time (date of sampling relative to parturition), and parity number on DM content and % Brix in urine and saliva were considered significant at $P < 0.05$. Relationships between % Brix and DM content in urine and saliva are indicated by the correlation coefficients (r) in panels E and F.

whereby the urea-N recycling mechanisms act as buffer for fluctuations in the dietary N supply (Lapierre and Lobley, 2001; Reynolds and Kristensen, 2008). During periods of low dietary N, cows can increase the arterial urea-N extraction across the ruminal wall and the portal-drained viscera (Kristensen et al., 2010). Additionally, the kidneys salvage urea-N by decreasing the renal urea-N clearance and by increasing the reabsorption of urea-N filtered by the kidneys (Kristensen et al., 2010; Røjen et al., 2011). Hence, in the present study, the lower blood urea concentrations observed in HO cows might be due to an increased urea uptake by the rumen and the portal-drained viscera. Though, in the case of an improved urea recycling capacity in HO cows, one would expect a concomitantly reduced renal urea excretion and possibly a greater urea concentration in saliva in HO compared with BS cows. However, the present results show that urea concentrations in saliva and urine do not differ between HO and BS. Furthermore, both breeds showed similar fecal nitrogen contents.

Urine and saliva samples were analyzed for their DM content and with a Brix refractometer indicating that Brix values reflects very closely DM in both media. In agreement with the comparable urea and creatinine concentrations in saliva of BS and HO cows, saliva DM was not affected by breed. Besides, we observed higher urea concentrations in saliva than in blood, while creatinine concentrations were similar in saliva and blood. Our findings are in contrast to previous studies conducted in heifers (Marini and van Amburgh, 2003), sheep (Piccione et al., 2006), dogs (Tvarijonaviciute et al., 2018) and humans (Venkatapathy et al., 2014; Lasisi et al., 2016) reporting lower urea concentrations in saliva than in blood. The discrepancy between our results and the experiment performed in heifers might be related on the one hand, to the fact that heifers are still growing ruminants and, on the other hand, the technique of saliva sampling (i.e., Salivette vs suction from the oral cavity). The urea transporter UT-B detected in the bovine parotid salivary gland (Dix et al., 2013) possibly enables an increased transfer of urea resulting in the observed up-concentration of urea in saliva compared with blood. Additionally, the fact that in humans UT-B transporters seem not to be present in the salivary gland (Sands, 2002) supports our hypothesis of elevated urea concentrations in bovine saliva via the UT-B transporter. On the other hand, we suggest that in cows creatinine is not transported actively into saliva since the creatinine concentrations in blood and saliva were similar.

A great variation in blood urea and creatinine concentrations between breeds was described in dogs (Drost et al., 2006; Zaldívar-López et al., 2011; Misbach et al., 2014), cats (Reynolds et al., 2010; Paltrinieri et al., 2014) and between human ethnicities (Jones et al., 1998) which were not due to differences in body weights or muscle mass. These findings were explained by differences in the glomerular filtration rate (GFR) between ethnicities and breeds (Jones et al., 1998; Zaldívar-López et al., 2011; von Hendy-Willson and Pressler, 2014). Recently, Müller et al. (2021) and Prahl et al. (2022) described high and low levels of milk urea in HO dairy cows fed the same diet with either low or normal crude protein concentrations. Cows showing high milk urea concentrations also had higher urea concentrations in blood, although urea content in urine did not differ between cows with low blood and milk urea. The authors speculated about lower renal clearance rate causing the differences in urea blood concentrations between cows. In cows, a study conducted by Murayama et al. (2014) revealed a higher GFR in dairy (Holstein) compared with beef (Japanese Black) cows. To investigate if the higher blood urea concentrations observed in BS compared with HO cows might be related to different GFR, we measured creatinine in blood, urine, and saliva as well as serum SDMA concentration in both breeds. Urine was collected by spot sampling since the creatinine excretion was shown to be constant during the day (Chizzotti et al., 2008). In accordance with the urea concentrations in blood, urine, and saliva, we observed higher creatinine concentrations in blood of BS cows, whereas creatinine concentrations in urine and saliva did not differ between breeds. Moreover, we did not observe breed differences in urine osmolality and urinary DM content. These results support the hypothesis of a different GFR in BS and HO cows. This assumption is further supported by the higher SDMA concentration in BS compared with HO. Symmetric dimethylarginine is a methylated amino acid (arginine) emanating from proteolysis and then released to the

Table 3. Fecal DM and nitrogen content in Brown Swiss (BS: n = 31) and Holstein (HO: n = 43) cows (mean ± SEM). Effects of breed (BS vs. HO), time (date of sampling relative to parturition), and parity number on fecal DM and nitrogen content were considered significant at $P < 0.05$

	Breed		*P*-values		
Parameter	BS	HO	Breed	Time	Parity
DM content (%)	13.28 ± 0.26	13.37 ± 0.26	$P = 0.59$	$P = 0.41$	$P = 0.35$
Nitrogen content (% in DM)	2.57 ± 0.06	2.64 ± 0.08	$P = 0.53$	$P = 0.59$	$P = 0.91$

circulation (Kakimoto and Akazawa, 1970; Hokamp and Nabity, 2016). Symmetric dimethylarginine is excreted by the kidney, thereby freely filtered by the glomerulus (MacAllister et al., 1996), but apparently not reabsorbed by the renal tubules (Kakimoto and Akazawa, 1970). Furthermore, SDMA is produced on a relatively constant rate. In small animal veterinary medicine SDMA is a useful endogenous GFR marker and an established indicator for decreased renal function, e.g., chronic kidney disease in cats and dogs (Hokamp and Nabity, 2016; Loane et al., 2022; Michael et al., 2022). As a biomarker of GFR, the greater serum levels of SDMA in BS than in HO point toward disparities in GFR between these breeds.

CONCLUSIONS

Under identical feeding conditions, BS had higher concentrations of urea, creatinine, and SDMA in blood compared with HO cows, while in urine and saliva urea and creatinine concentrations did not differ between breeds. These results point toward a lower glomerular filtration rate in BS as in HO cows. The up-concentration of urea in saliva compared with the level in blood might be related to the presence of urea transporter UT-B in the bovine parotid salivary gland while the transfer of creatinine into saliva seems to occur passively. However, estimation of nitrogen excretion via milk, urine, and feces does not entirely reflect nitrogen turnover within the animal.

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