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Metabolic adaptation to energetic demands of early lactation in Holstein Friesian and Simmental cows

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ABSTRACT

Holstein Friesian (**HF**) is the highest-performing dairy breed worldwide. However, this breed is prone to suffer from health disorders and infertility due to metabolic instability. The aim of the study was to use the metabolic differences between to breed, German HF and German Simmental (**SI**), for discussion of the biological impact of well-known and novel pathways and their respective indicators. Therefore, a targeted metabolomics approach combined with an examination of classical clinical chemistry using plasma samples was used to describe the metabolic status 42 days before expected calving and at day 21 of lactation. In total, 27 multiparous cows (HF n = 17, SI n = 10) were enrolled in the study. All cows were kept on the same farm under identical management, feeding and housing conditions. BW of HF cows dropped while SI cows maintained their BW but produced less milk than HF cows during the first weeks of lactation. Differences were detected for the following indicators and related pathways, and were used to assess their biological impact. Branched-chain amino acids such as leucine and isoleucine at day +21 remained as high as at day −42 in HF cows; however, in SI cows, a clear drop in these amino acids was observed indicating less body protein mobilisation in this breed. Alpha amino-adipic acid and kynurenine as markers for oxidative stress balance were increased at day +21 in HF cows, only reflecting a metabolically more tensed condition in HF cows. As a conclusion, these findings confirm the impact of these metabolites on a higher risk to suffer from metabolic disorders.

Implications

Farmers, consumers and scientists are aware of dairy cow welfare issues. High-yielding dairy cows are prone to suffer from production diseases. To improve cows' health, knowledge about resource allocation pathways is needed. This trial attempted to detect novel indicators of pathways associated with the onset of lactation in a dairy breed, Holstein Friesian, and a dual-purpose dairy breed, Simmental. The major finding was that Holstein Friesian and Simmental intriguingly differed in their metabolism of branched–chain amino acids. This might indicate a higher protein turnover in Holstein Friesian. Since branched chain amino acids are known to reflect a more tensed metabolic condition in other species, the results of the present study point to these metabolites as a potential topic for further research about metabolic stability in dairy cows.

Introduction

Throughout the lactation cycle, negative energy balance during transition and early lactation is one challenge high-performing dairy cows are faced with. Animals counteract with mobilisation of body fat and protein reserves accompanied by modifications of insulin effects and glucose metabolism (Bauman and Currie, 1980; Herdt, 2000; Hayirli, 2006; Bruckmaier and Gross, 2017). Adaptive response to negative energy balance in dairy cows is reflected by body condition loss, lowered glucose concentrations and elevated concentrations of non-esterified fatty acids (**NEFA**) and β-hydroxybutyrate (**BHBA**) (Herdt, 2000; Schröder and Staufenbiel, 2006; Roche et al., 2009; Pires et al., 2013). The capacity to cope with intrinsic and extrinsic challenges maintaining homeorhesis in a long run could be termed as metabolic stability. Metabolic stability can be disrupted in the individual cow resulting

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in poor adaptation to negative energy balance indicated by high plasma concentration of NEFA and BHBA above the thresholds for subclinical or clinical ketosis. Poor adaptation to negative energy balance (Duffield et al., 2009; McArt et al., 2012; McArt et al., 2013) is further associated with higher susceptibility for disease and lower productive performance (Drackley, 1999; Ospina et al., 2013). Pure dairy breeds selected for high milk yields in general are considered less metabolically stable than dual–purpose breeds as assessed by modelling the repeatability of feed intake, energy balance and milk yield during days in milk (Harder et al., 2019). Furthermore, differences in the dynamics of body condition and plasma concentrations of NEFA and BHBA have been reported between Simmental (**SI**) cows, a common dual-purpose breed and Holstein Friesian (**HF**) cows, a genetically strictly selected dairy breed. In early lactation, HF cows lost more body condition and displayed higher concentrations of NEFA and BHBA in blood than SI cows (Lopreiato et al., 2019; Knob et al., 2021; Strączek et al., 2021). Differences in metabolic adaptation to transition between SI and HF cows were identified to be related to energy metabolism, inflammation response and liver function (Lopreiato et al., 2019; Lopreiato et al., 2020). The most evident difference between SI and HF cows is the relatively higher proportion of skeletal muscle weight in the carcasses of SI cows (Gallo et al., 2017). Musculature is a metabolically quite active organ that can use different substrates such as NEFA and BHBA as energetic fuels and provides amino acids for various metabolic pathways including hepatic gluconeogenesis. Pires et al. (2013) suggested higher muscle protein mobilisation in HF cows during negative energy balance. A direct association between skeletal muscle mass, protein mobilisation and ketogenesis has been proposed in dairy cattle (Akers, 2006; van der Drift et al., 2012). Moreover, the musculature might play an important role in the development of insulin resistance, at least in HF cows (Sadri et al., 2020).

Metabolomics is a well-known approach to examine and characterise metabolism under certain conditions that already has been widely applied to dairy cows (Kenéz et al., 2016; Schären et al., 2021a; Schären et al., 2021b). The present study aimed to compare HF and SI cows under identical feeding, management and housing conditions in an anabolic state at dry off (day –42 relative to calving) and in a catabolic state at day +21 of lactation. It was hypothesised, that based on already known differences between the HF and SI breeds, remarkable differences exist in potentially underlying intermediary pathways related to energy and substrate metabolism during the onset of milk production. Using a targeted metabolomics approach, novel findings about specific metabolic pathways aimed to better understand in–depth breed-specific metabolic traits. Correlation of metabolites of interest with classical performance and clinical biochemical data allows to interpret novel findings and identify pathways, which characterise metabolic differences between HF and SI cows.

Material and methods

Animals, feeding and management

Holstein Friesian (HF, n = 17) and German Simmental (SI, n = 10) entering second lactation or higher were enrolled in the study between September 2016 and July 2017. Cows of both breeds were kept under the same prepartum and postpartum conditions, housed in a freestall barn at the Livestock Centre of the Faculty of Veterinary Medicine, LMU Munich, Germany. Cows were dried off approximately 8 weeks before the expected calving date and kept in a separate dry cow freestall barn. Within 1 week before the expected calving date, cows were moved into a tie-stall facility that was used as a maternity pen. Cows were reintegrated into the lactating herd around days six to eight after parturition. Cows were fed a total mixed ration based on grass and corn silage *ad libitum*. During the dry period, rations were formulated according to the recommendations of the German Society of Nutrition Physiology (GfE, 2001). Transition feeding started 2 weeks prior to calving with increasing amounts of concentrate (7.2 MJ NEL/kg) ending up with two kg per day. Lactating cows additionally received transponder-controlled individual concentrate allocation (7.8 MJ NEL/kg) adjusted to their individual milk yield. Details on components and nutritive values of the diet are given in Table 1. The diet was offered twice daily. Concentrate allocation was restricted to a maximum intake of 8 kg per day. During the first 30 days in milk, 150 ml of propylene glycol and glycerol (ProGlyc®, VILOFOSS, Neuenkirchen-Vörden, Germany) per day were added to the ration. All cows had *ad libitum* access to water.

Assessment of production data

The farm uses an automated milking system recording individual milk yield and BW on a daily basis (Lely Astronaut A3 and Lely Astronaut A3 Next, Lely, Waldstetten, Germany). Furthermore, the local controlling agency ("LKV Bavaria" – Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V, Munich, Germany) surveyed milk contents on the basis of monthly milk performance test recordings. BW was pooled for each week from days 8 to 28 of lactation (BW_{wk2} to BW_{wk4}). First milk recording for this study took place within the beginning of the third and the beginning of the fifth week of lactation. Thus, milk yield was cumulated for the third and fourth week of lactation. Based on milk content data provided by LKV Bavaria, the caloric value of milk (**LE**) was calculated according to the recommendations of the GfE (2023).

$$\mathrm{LE}\ (\mathrm{kJ/kg}) = 38.5 * \mathrm{fat}\ (\mathrm{g/kg}) + 24.2 * \mathrm{protein}\ (\mathrm{g/kg}) + 16.5 * \mathrm{lactose}\ (\mathrm{g/kg}).$$

Subsequently, energy output via milk (E_{out} milk) was the product of LE and milk yield.

Sampling and analysis of blood samples

Cows were sampled about 42 days before the expected date of calving (d –42) and 21 days after parturition (d +21). Body condition scoring was performed at day –42 and day +21 according to Edmonson et al. (1989). Blood samples were taken before morning feeding by venipuncture into commercially available lithium heparin and serum tubes (Sarstedt AG, Nümbrecht, Germany). After collection, blood samples were immediately cooled and processed within 15 min. Plasma and serum aliquots were stored at –80 °C until further biochemical analysis. Clinical chemical parameters were measured in serum using an automatic analyzer (Cobas c 311, Roche Diagnostics, Mannheim, Germany). Plasma concentrations of insulin were determined using a commercially available bovine ELISA kit (Cat.No.: 10-1201-01, Mercodia, Uppsala, Sweden). Surrogate indices for insulin sensitivity RQUICKI (Perseghin et al., 2001; Holtenius and Holtenius, 2007; Goerigk et al., 2011) and RQUICKI-BHBA (Balogh et al., 2008) were calculated.

Targeted metabolomics

The targeted metabolomics approach was performed in plasma using the AbsoluteIDQ p180 Kit (Biocrates Life Science AG, Innsbruck, Austria) according to the manufacturer's protocol. This kit identifies up to 188 metabolites belonging to five compound classes such as acylcarnitines, proteinogenic and modified amino acids, biogenic amines and glycerophospho- and sphingolipids (**PC, SM**). Hexoses were also measured. In brief, samples, standards

Table 1
Contents and nutrient composition of the diet fed to Holstein Friesian and Simmental cows before and after parturition.

Item	Total mixed ration	
	Lactation feeding	Dry cow feeding
Contents (% DM)		
Maize silage	41.7	22.4
Grass silage	29.7	60.1
Barley straw	1.70	15.9
Barley grain	2.85	
Wheat grain	2.85	
Grain corn	6.27	
Rapeseed meal	4.56	
Soybean meal	8.54	
Molasses (sugar beet)	1.14	
Limestone[1]	0.11	
Mineral mix[2,3]	0.57	0.93
Salt	0.17	
Chemical analysis		
CP [g/kg DM]	146.9	107.6
Crude ash [g/kg DM]	69.1	91.1
Ether extract [g/kg DM]	29.4	30.7
Crude fibre [g/kg DM]	179.4	275.8
NFE [g/kg DM]	575.2	494.7
NEL [MJ/kg DM]	6.7	5.6
	Concentrate feeding	
	Concentrated [standard]	Concentrate [energy]
Contents (% FM)		
Barley grain	15.40	10.13
Wheat grain	15.50	20.57
Grain corn	25.50	31.90
Rapeseed meal	24.00	11.0
Soybean meal	18.00	20.4
Limestone	1.20	
Salt	0.40	
Soybean oil		1.0
Yeast concentrate[4]		2.0
Rumenstable fat[5]		3.0
Chemical analysis (per kg FM)		
DM [g]	893	897.9
NEL [MJ]	7.2	7.8
CP [g]	210.2	185.4
Crude fibre [g]	51	39.8
Crude fat [g]	26.8	59.3
Starch + sugar [g]	406.9	450.7
Calcium [g]	7.1	6.6
Phosphorus [g]	5.6	4.4
Magnesium [g]	2.2	1.9
Sodium [g]	1.6	1.9
Potassium [g]	8.8	7.6
Mineral mix content [g]	8.0	20

Abbreviations: FM = fresh matter; NFE = nitrogen free extract fraction; NEL = net energy lactation
[1] Calcium carbonate.
[2] SALVANA ZELLmin
[3] SALVANA TR -40/PRENATAL-E.
[4] SALVANA AKTIV HEFE.
[5] SALVANA PROFEED-FAT (Salvana Tiernahrung GmbH, Klein-Offenseth-Sparrieshoop, Germany).

and controls were applied onto the matrix of the multititer plate of the kit and subsequently dried under nitrogen flow. Dried samples were derivatised with 5% phenylisothiocyanate and extracted with ammonium acetate in methanol. Amino acids and biogenic amines were analysed by liquid chromatography – mass spectrometry, whereas for the remaining analytes, flow injection analysis-mass spectrometry was used. Liquid chromatography and mass spectrometry were carried out at the Core facility of the University of Hohenheim (Stuttgart, Germany). For further details concerning the analytical procedure, see Bäßler et al. (2021) and Kenéz et al. (2016).

Table 2
Milking performance data (least square means ± SEM) of the current study lactation in Holstein Friesian and Simmental dairy cows.

Item	Holstein cows (n = 17)	Simmental cows (n = 10)	*P*-Value
Lactation number	3.4 ± 0.5	4.2 ± 0.6	0.30
DIM milk recording	22.8 ± 3.0	24.2 ± 3.9	0.78
Fat %	3.9 ± 0.2	3.7 ± 0.2	0.40
Protein %	3.4 ± 0.10	3.3 ± 0.13	0.69
Lactose %	4.8 ± 0.04	4.8 ± 0.05	0.43
LE (MJ/kg milk)	3.13 ± 0.08	3.03 ± 0.10	0.46

Abbreviations: DIM = days in milk; LE = energy content of milk.

Statistical analysis

Statistical analysis for performance data and classical clinical chemistry was performed using the JMP® Pro 17 Software (JMP Statistical Discovery LLC, Cary, NC, USA). All variables were tested for normal distribution by Shapiro-Wilk test. BCS was tested for significant differences between d −42 and d +21 within each breed by using a paired *t*-test. A two-way ANOVA for repeated measures was performed in order to assess the effects of time and breed including their interaction effects on data collected during the current dry period and during the first 4 weeks of lactation. Multiple comparisons were performed using Tukeýs HSD posthoc test. $P \leq 0.05$ was considered significant.

Bioinformatical evaluation of metabolite profiles

Differences of plasma metabolite profiles over time and between breeds were determined using the open-source software MetaboAnalyst 6.0 (https://www.metaboanalyst.ca; Pang et al., 2024). After log transformation and Pareto scaling, data were normalised. Partial Least Squares (**PLS**)-Discriminant Analyses (**DA**) were performed to visualise differences between breeds at the two sampling time points. The predictive ability of the model was estimated via permutation test and cross–validation. Metabolites with a variable importance projection score of 1.5 or higher on day +21 were then subjected to the same statistical procedure as the classical variables (see above). Components with a variable importance projection score higher than 1.5 on day +21 and a *P*-value ≤ 0.05 for main effects or interactions of time and breed were chosen as metabolites of interest. Metabolites of interest were correlated with production data as well as with indicators of energy metabolism and insulin sensitivity using Pearsońs correlations.

Results

Milk yield, milk composition and concentrate allocation

Milk yield was significantly influenced by lactation week (time) and breed. Lactation number, days in milk and milk contents at first milk recording did not differ between breeds (Table 2). Milk yield was similar in lactation week four compared to lactation week 3 in both breeds, but SI cows consistently yielded less milk than HF cows. There were no differences in LE between the breeds, so E_{out}milk was higher in HF cows than in SI cows in both weeks of lactation (Table 3). Concentrate allocation via the automatic feeder at least until day +21 did not differ (Mean ± SEM: HF: 67.7 ± 1.8 kg vs SI: 66.3 ± 3.4 kg; $P = 0.70$).

BW and body condition

Cows at both sampling days were in adequate condition for the respective production phase and breed. In both breeds, body condition score at day +21 (Mean ± SEM: HF: 2.79 ± 0.12; SI: 3.2 ± 0.14) was significantly lower than at day −42 (Mean ± SEM: HF: 3.43 ± 0.12; SI: 3.85 ± 0.14). After calving, BW was maintained in SI cows within the second to fourth week of lactation whereas HF cows had lower BW in lactation week four compared to lactation week two and lactation week three (Fig. 1).

Clinical blood chemistry and surrogate indices of insulin sensitivity

Time exerted a significant effect on glucose, BHBA, insulin and creatinine. Glucose, insulin and creatinine concentrations were lower at day +21 of lactation than at day −42, whereas BHBA was higher. Breed significantly influenced BHBA, urea, creatinine and γ-glutamyl transferase with lower concentrations of BHBA, lower activity of γ-glutamyl transferase and higher concentrations of creatinine and urea in SI cows. A significant interaction of time and breed could not be shown for any of the variables tested. Details on clinical chemistry and surrogate indices of insulin sensitivity are given in Table 4.

Metabolite profiling

Partial Least Squares-Discriminant Analysis at day −42 before parturition revealed an only weak separation between HF and SI cows (data not shown). At day +21, PLS-DA revealed eight amino acids, creatinine, eleven long-chain PC and three SM, one acylcarnitine and three biogenic amines with a variable importance projection score > 1.5 (Fig. 2 A, B). However, the validity of the model was also weak as indicated by low Q2 values and failure of the permutation test (Fig. 2 C, D). Subsequent Two-Way ANOVA showed significant effects of time (day −42, day +21), breed (HF, SI) or interactions between time and breed for 21 of these variables. Details are given in Table 5).

Amino acids, amino acid derivates and biogenic amines

All amino acids identified as metabolites of interest were influenced by breed and with the exception of arginine and aspartate also by time, whilst an interaction of time and breed could not be shown for any of these amino acids. In general, concentrations of amino acids were lower or equal at day +21 in comparison to day −42 except for asparagine and proline that showed an inverse pattern. Furthermore, concentrations of circulating amino acids were consistently lower in SI cows than in HF cows at day +21. Only lysine concentration was also lower in SI cows at day −42 compared to HF cows (Table 5). In HF cows, lysine was the only amino acid which decreased between day −42 and day +21.

There was a significant interaction between time and breed for α-amino-adipic acid (**α-AAA**), with significantly higher concentrations at day +21 compared to day −42 in HF cows, whilst concentrations in SI cows did not differ within the sampling days.

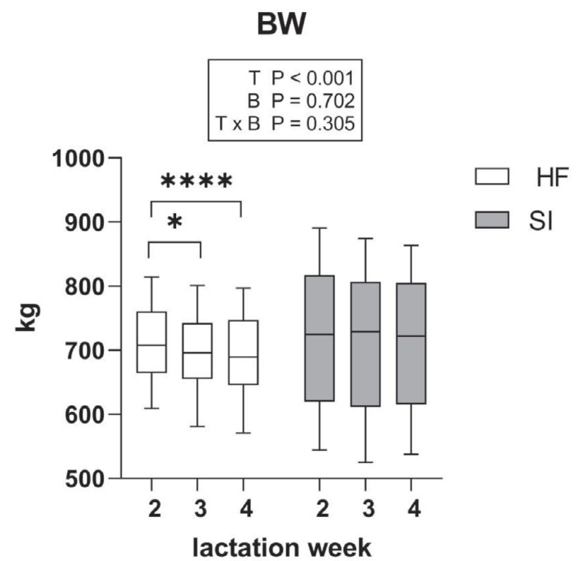

Fig. 1. Changes in BW were demonstrated over the first four lactation weeks in Holstein Friesian and Simmental cows in box-and-whisker diagrams. The box plot shows the median (50th), 25th, 75th percentiles and minimum/maximum (0th, 100th percentiles). Two–Way ANOVA revealed a significant effect of time with decreased BW in HF cows only. * $P \leq 0.05$, **** $P \leq 0.0001$; Tukeýs HSD posthoc test. Abbreviations: HF = Holstein Friesian; SI = Simmental; T = time; B = Breed.

Kynurenine concentrations expressed an increase from day −42 to day +21 in HF cows only as reflected by a significant interaction between time and breed. Sarcosine concentrations were lower at day +21 compared to day −42 in HF cows only. SI cows generally showed higher sarcosine concentrations than HF cows (Table 5).

Phosphatidylcholines and lyso-phosphatidylcholines

Among metabolites of interest, PC with a chain length of ≥ C28 were significantly influenced by time, breed or both. Most PC aa were influenced by time and one third of them also by breed with SI cows showing generally lower concentrations compared to HF cows. A significant interaction of both factors could also be shown for certain PC. Concentrations of Lyso PC a 16:1 were significantly influenced by time, but no breed effect or interaction could be shown. Details on statistics are given in Table 5.

Acylcarnitines and sphingomyelines

Sphingomyelines and Acylcarnitines were sparsely influenced by time or breed. Significantly higher concentrations in HF cows compared to SI cows could be shown for SM C 24:1 and SM(OH) C 22:1. Time exerted a significant effect on SM(OH) C 22:1 with higher concentrations at day +21 in both breeds. Short-chain acylcarnitines glutaconylcarnitine (C5:1DC) was found in slightly higher concentrations at day +21 in SI cows compared to HF cows. (Table 5).

Table 3
Milk yield and energy output (least square means ± SEM) in the study lactation in Holstein Friesian and Simmental cows.

Item	Holstein cows (n = 17)		Simmental cows (n = 10)		*P*-Value		
	LW 3	LW 4	LW 3	LW 4	T	B	T × B
MY (kg)	301 ± 9.97[a]	321 ± 9.97[b]	246 ± 13.0[c]	274 ± 13.0[a]	<0.001	0.003	0.38
E_{out}milk(MJ)	940 ± 31.8[a]	998 ± 31.8[b]	740 ± 41.5[c]	826 ± 41.5[a]	<0.001	0.001	0.38

Abbreviations: LW = lactation week; T = time; B = breed; MY = milk yield; E_{out}milk = Energy output via milk.
Values within a row with different superscripts differ significantly at $P \leq 0.05$.

Table 4
Clinical blood chemistry and surrogate indices for insulin sensitivity (least square means ± SEM) in Holstein Friesian and Simmental cows 42 days before expected calving date and at day +21 of lactation.

	Holstein (n = 17)		Simmental (n = 10)		*P*-Value		
Item	d −42	d +21	d −42	d +21	T	B	T × B
Glucose (mmol/L)	3.92 ± 0.08[a]	3.62 ± 0.08[b]	3.81 ± 0.10[ab]	3.64 ± 0.10[ab]	0.002	0.625	0.334
NEFA (mmol/L)	0.33 ± 0.08	0.64 ± 0.08	0.42 ± 0.10	0.42 ± 0.10	0.128	0.429	0.135
BHBA (mmol/L)	0.47 ± 0.05[a]	0.74 ± 0.05[b]	0.38 ± 0.07[a]	0.53 ± 0.07[ab]	0.001	0.022	0.283
Insulin (mU/L)	13.8 ± 1.5[a]	6.70 ± 1.5[b]	10.3 ± 1.9[ab]	8.65 ± 1.9[ab]	0.014	0.659	0.109
RQUICKI	0.44 ± 0.01	0.44 ± 0.01	0.44 ± 0.02	0.47 ± 0.02	0.352	0.282	0.345
RQUICKI-BHBA	0.52 ± 0.02	0.47 ± 0.02	0.54 ± 0.03	0.56 ± 0.03	0.496	0.053	0.266
Total protein (g/L)	78.7 ± 1.40	77.8 ± 1.40	76.0 ± 1.84	75.2 ± 1.84	0.315	0.218	0.975
Albumin (g/L)	36.7 ± 0.9	36.3 ± 0.9	37.0 ± 1.10	35.2 ± 1.10	0.214	0.724	0.444
Globulin (g/L)	42.0 ± 1.84	41.5 ± 1.84	39.0 ± 2.40	39.9 ± 2.40	0.846	0.397	0.599
Bilirubin (µmol/L)	1.5 ± 0.27	1.96 ± 0.27	1.48 ± 0.33	1.52 ± 0.33	0.352	0.479	0.437
Urea (mml/l)	2.51 ± 0.22[a]	3.42 ± 0.22[abc]	3.58 ± 0.29[b]	3.71 ± 0.29[c]	0.091	0.002	0.200
Creatinine (µmol/L)	82.5 ± 4.24[a]	72.6 ± 4.24[a]	107 ± 5.53[b]	102 ± 5.53[b]	0.031	< 0.001	0.503
AST (U/L)	83.6 ± 4.0	83.1 ± 4.0	75.0 ± 5.16	83.7 ± 5.16	0.350	0.424	0.287
GLDH (U/L)	20.3 ± 3.44	18.5 ± 3.44	11.0 ± 4.49	16.5 ± 4.49	0.470	0.277	0.224
GGT (U/L)	25.8 ± 2.12	27.8 ± 2.12	20.8 ± 2.77	21.7 ± 2.77	0.492	0.049	0.821
CK (U/L)	327 ± 84.6	109 ± 84.6	115 ± 110	114 ± 110	0.262	0.313	0.269

Abbreviations: T = time; B = breed; NEFA = non-esterified fatty acids = BHBA: beta-hydroxybutyrate; RQUICKI = revised insulin sensitivity check index; RQUICKI-BHBA = revised insulin sensitivity check index including BHBA; AST = aspartate amino transferase; GLDH = glutamate dehydrogenase; GGT = gamma glutamyl transferase; CK = creatinine kinase.
Values within a row with different superscripts differ significantly at $P \leq 0.05$.

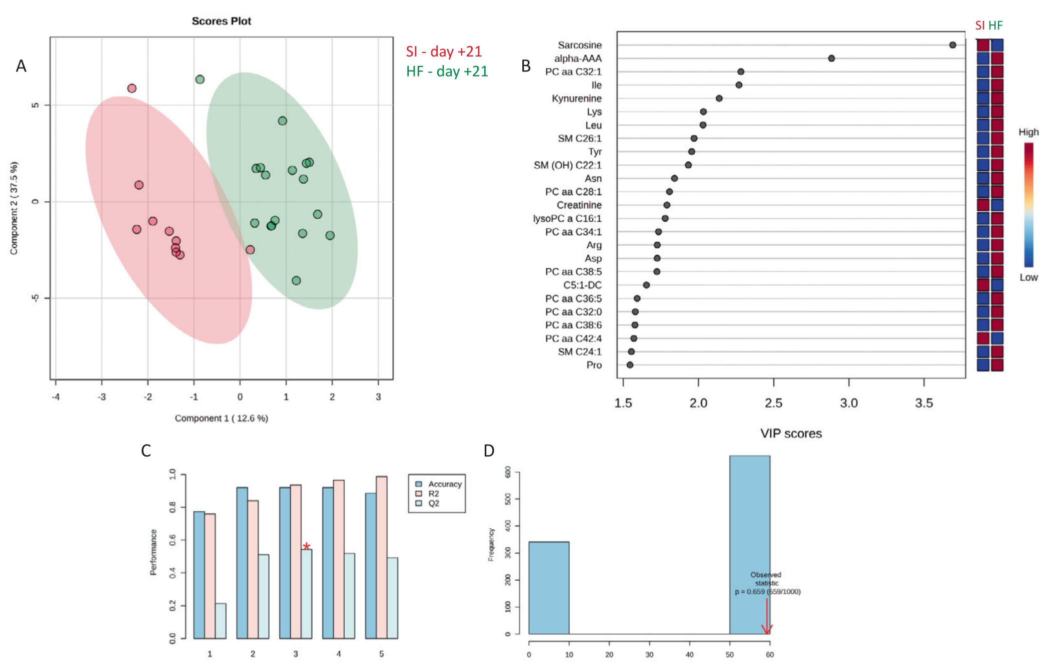

Fig. 2. (A) Partial least squares-discriminant analysis demonstrated two distinct groups at day +21 postpartum; green Holstein Friesian cows, red Simmental cows. Components 1 and 2 explain 49.7% of the variation. (B) Variable of importance in projection (VIP scoring) was done for 25 metabolites with a score > 1.5. Validity of the model was proven by cross–validation (C) and permutation test (D). Red asterisk indicates the model with the highest Q2 value. Abbreviations: HF = Holstein Friesian; SI = Simmental.

Correlations

Significant strong correlations at day +21 could be found among amino acids, but especially the branched chain amino acids leucine and isoleucine as well as lysine further correlated positively with indicators of energy metabolism (NEFA, BHBA) and negatively with surrogate indices for insulin sensitivity (RQUICKI, RQUICKI-BHBA) (Table 6).

Table 5
Metabolites of interest (least square means ± SEM) as identified by partial least squares discriminant analysis (PLS-DA) from metabolite profiling (IDQ p180 kit, Biocrates, Austria) in Holstein Friesian and Simmental cows 42 days before expected calving date and at day +21 of lactation.

Item (μmol/l)	Holstein (n = 17)		Simmental (n = 10)		*P*-value		
	d −42	d +21	d −42	d +21	T	B	T × B
Amino acids							
Arginine	70.2 ± 3.09[a]	66.1 ± 3.09[a]	59.1 ± 4.00[ab]	51.7 ± 4.00[b]	0.122	0.002	0.651
Asparagine	28.4 ± 1.68[ac]	45.4 ± 1.68[b]	23.7 ± 2.18[a]	35.2 ± 2.18[c]	<0.001	0.002	0.140
Isoleucine	107 ± 5.51[a]	105 ± 5.51[a]	96.0 ± 7.20[ab]	71.2 ± 7.20[b]	0.050	0.001	0.106
Leucine	133 ± 6.27[a]	115 ± 6.27[a]	120 ± 8.20[a]	82.5 ± 8.20[b]	<0.001	0.006	0.162
Lysine	99.8 ± 3.90[a]	78.5 ± 3.90[c]	78.9 ± 5.10 [bc]	60.1 ± 5.10[b]	<0.001	<0.001	0.808
Tyrosine	50.8 ± 2.10[a]	44.9 ± 2.10[a]	43.8 ± 2.75[a]	33.3 ± 2.75[b]	<0.001	0.002	0.321
Proline	65.4 ± 2.83[a]	88.9 ± 2.83[c]	60.5 ± 3.69[a]	73.7 ± 3.69[b]	<0.001	0.009	0.107
Amino acid derivates and biogenic amines							
α-AAA	1.90 ± 0.14[a]	2.89 ± 0.14[b]	1.77 ± 0.18[a]	1.73 ± 0.18[a]	0.002	0.001	0.001
Kynurenine	5.83 ± 0.48[a]	7.53 ± 0.48[b]	5.81 ± 0.63[ab]	5.08 ± 0.63[a]	0.246	0.081	0.006
Sarcosine	0.91 ± 0.07[a]	0.58 ± 0.07[b]	1.17 ± 0.10[a]	1.08 ± 0.10[a]	0.021	<0.001	0.167
Phosphatidylcholines and lyso-phosphatidylcholines							
PC aa C 28:1	1.84 ± 0.15[a]	2.64 ± 0.15[b]	1.54 ± 0.19[a]	1.96 ± 0.19[a]	<0.001	0.026	0.134
PC aa C 32:0	8.08 ± 0.36[a]	6.75 ± 0.36[b]	6.77 ± 0.48[ab]	5.50 ± 0.48[bc]	<0.001	0.023	0.914
PC aa C 32:1	8.32 ± 0.61[a]	12.1 ± 0.61[b]	7.37 ± 0.79[a]	8.50 ± 0.79[a]	<0.001	0.002	0.020
PC aa C 34:1	96.7 ± 6.86[a]	132 ± 6.86[b]	89.4 ± 8.95[a]	105 ± 8.95[ab]	<0.001	0.087	0.099
PC aa C 36:5	9.53 ± 0.61[a]	9.00 ± 0.61[ab]	9.29 ± 0.80[a]	6.75 ± 0.80[b]	0.010	0.150	0.079
PC aa C 38:6	3.48 ± 0.23	4.10 ± 0.23	3.46 ± 0.29	3.19 ± 0.29	0.425	0.130	0.049
PC aa C 42:4	0.11 ± 0.01[a]	0.08 ± 0.01[b]	0.09 ± 0.01[ab]	0.09 ± 0.01 [ab]	0.002	0.430	0.110
Lyso PC a C 16:1	1.12 ± 0.08[a]	1.53 ± 0.08[b]	0.99 ± 0.10[a]	1.19 ± 0.10[ab]	<0.001	0.057	0.079
Sphingomyelines							
SM C 24:1	5.37 ± 0.26	5.13 ± 0.26	4.21 ± 0.34	4.28 ± 0.34	0.773	0.008	0.574
SM(OH) C 22:1	8.62 ± 0.59[ab]	12.4 ± 0.59[c]	7.03 ± 0.77[b]	9.39 ± 0.77[a]	<0.001	0.010	0.187
Acylcarnitines							
C5:1 DC	0.03 ± 0.001[ab]	0.03 ± 0.001[a]	0.03 ± 0.001[ab]	0.04 ± 0.001[b]	0.592	0.020	0.132

Abbreviations: T = time; B = breed; α-AAA = α-amino adipic acid; PC = phosphatidylcholines; SM = sphingomyelins; C5:1 DC = glutaconylcarnitine.
Values within one row with different superscripts differ significantly at $P \le 0.05$.

Table 6
Pearsońs correlation coefficients of linear relationships between plasma amino acid concentrations and indicators of energy metabolism and surrogate indices of insulin sensitivity in Holstein-Friesian (n = 17) and Simmental (n = 10) cows at day 21 of lactation.

Item	Asparagine	Isoleucine	Leucine	Lysine	Tyrosine
Arginine	0.61 ***	0.29	0.56**	0.84***	0.80***
Asparagine	–	0.25	0.29	0.60**	0.73***
Aspartate	−0.05	−0.15	−0.12	−0.08	0.13
Isoleucine	0.25	–	0.90***	0.55**	0.23
Leucine	0.29	0.90***	–	0.72***	0.43*
Lysine	0.60**	0.55**	0.72***	–	0.76***
Proline	0.79***	0.28	0.39*	0.75***	0.71***
	Glucose	NEFA	BHBA	RQUICKI	RQUICKI-BHBA
Arginine	0.05	−0.13	−0.14	−0.26	−0.12
Asparagine	0.21	−0.10	−0.21	0.05	0.08
Aspartate	−0.51**	−0.23	0.27	0.17	−0.05
Isoleucine	0.22	0.49*	0.60**	−0.41*	−0.51**
Leucine	0.25	0.34*	0.41*	−0.49**	−0.51**
Lysine	0.19	0.12	0.03	−0.47*	−0.40*
Proline	0.08	−0.001	−0.23	−0.15	−0.06

*$P < 0.05$, ** $P < 0.01$ *** $P < 80.001$.
Abbreviations: NEFA = non-esterified fatty acids; BHBA = beta-hydroxybutyrate; RQUICKI = revised insulin sensitivity check index; RQUICKI-BHBA = revised insulin sensitivity check index including BHBA.

Discussion

This study aimed to compare HF cows as a dairy breed to SI cows as a dual-purpose breed under identical feeding, housing, and management conditions at the early onset of lactation. Day −42 was chosen as a metabolically more stable condition, while at day +21, metabolic conditions are reflecting the onset of lactation. Acute metabolic disturbances due to parturition were not the focus of the study. One of the major limitations of this experimental approach was that individual total feed intake could not be measured. It is assumed that concentrate intake was equal in both breeds; however, milk output was lower in SI cows compared to HF cows during the recorded time period.

Classical assessment of metabolic status in Holstein Friesian and Simmental dairy cows during onset of lactation

In the present study, significantly lower concentrations of glucose and insulin and significantly higher concentrations of BHBA at day +21 compared to day −42 in HF cows might indicate a lower insulin efficacy and a less efficient utilisation of NEFA. Surrogate indices, RQUICKI (Holtenius and Holtenius, 2007), and a further modified index including BHBA (RQUICKI-BHBA) as proposed by Balogh et al. (2008) were used to assess insulin sensitivity. At least a strong tendency for an overall effect of breed on RQUICKI-BHBA could be shown possibly indicating lower insulin sensitivity in HF cows. Furthermore, the significant decline in insulin concentrations at day +21 contributed to a diminished insulin efficacy in HF cows. In SI cows, glucose and insulin concentrations were maintained during the onset of lactation, and NEFA and BHBA concentrations were also not affected. This metabolic status of SI cows represented a balanced adaptation to the onset of lactation which is indicative of a better developed metabolic stability compared to HF cows. As assessed by creatinine as an established marker for muscle mass (Megahed et al., 2019), SI cows had a higher muscle mass than HF cows consistently during the onset of lactation. It is well documented that SI cows had a higher proportion of muscle than HF cows (Gallo et al., 2017), and had higher creatinine concentrations in plasma (Lopreiato et al., 2019). Since muscle is a metabolically important tissue also in cattle (Sadri et al., 2023), the higher muscle mass might provide a healthier and more stable condition in SI cows. Besides signs of less efficient NEFA utilisation, HF cows lost BW at the onset of lactation most likely due to the higher output of milk energy, but also due to a more instable metabolic condition compared to SI cows.

Adaptation of metabolite profiles in Holstein Friesian and Simmental dairy cows to the onset of lactation

Amino acids, amino acid-related metabolites and biogenic amines

Concentrations of seven amino acids, such as arginine, asparagine, isoleucine, leucine, lysine, tyrosine and proline, were identified as influenced by the onset of lactation and breed. In SI, arginine, isoleucine, leucine, lysine and tyrosine decreased at day +21 compared to day −42, and asparagine and proline increased. In HF cows, only asparagine and lysine decreased at day +21 compared to day −42, but arginine, isoleucine, leucine and tyrosine did not. Asparagine and proline increased in this period like in SI cows.

One of the major findings is the lack of decrease in branched-chain amino acids (**BCAA**) isoleucine and leucine in HF cows. The concentration of a certain metabolite in blood is always a function of resorption from the gut, release from tissues or usage in catabolic or anabolic pathways. Since at least the concentrate allocation did not differ between breeds, and feeding conditions were similar, resorption from gut and modulation by the liver seemed to be of less importance for the differences in amino acid concentrations between the breeds found in the present study. Therefore, plasma amino acid concentrations at early lactation in HF and SI cows might be most likely balanced between influx from muscle tissues (Cant et al., 2022) and efflux to the mammary gland for milk protein synthesis (Chagas et al., 2007; Webb et al., 2019). An influence of time relative to parturition on concentrations of circulating BCAA has already been described for dairy cattle by Kenéz et al. (2016), Schären et al. (2021a) and Ghaffari et al. (2019). Elevated circulating BCAA were considered to be linked with insulin resistance since decades in humans and rodents (Felig et al., 1969) and this is also discussed for dairy cattle (Jorge-Smeding et al., 2021; Ghaffari et al., 2019). BCAA might favour reduced insulin sensitivity by over-stimulation of the mTOR pathway, thereby leading to an over-phosphorylation of the insulin receptor substrate, subsequently blunting insulin signalling on the cellular level. This might lead to enforced lipolysis with NEFA release and increasing ketone body formation. (Jorge-Smeding et al., 2021). The hypothesis of a negative relationship between insulin sensitivity and BCAA in dairy cattle is supported by significant correlations of leucine and isoleucine with NEFA and BHBA as well as with surrogate indices for insulin sensitivity in the present study. Insulin is known to favour BCAA oxidation in muscle, to potentially inhibit proteolysis and to increase BCAA clearance by the mammary gland (Nichols et al., 2016; Blair et al., 2021; Neinast et al., 2019, Qiao et al., 2024). As mentioned before, insulin concentrations decreased strongly after calving in HF cows compared to SI cows in the present study, thereby further contributing to lower insulin effects. Taken together, these findings indicated a more insulin-insensitive status in HF cows compared to SI cows leading to lower clearance of BCAA from plasma by muscle protein turnover and milk protein synthesis. Due to the molecular effects of leucine in terms of inhibition of insulin signalling cascade, stable BCAA concentrations in HF might maintain an insulin-insensitive status longer than in SI, thereby worsening the metabolic condition during the adaptation to onset of lactation. The increase in asparagine and proline concentrations in both breeds, but on a higher level in HF cows, indicated a change in the glutamate-asparagine-proline metabolism which is a modulatory pathway of protein turnover (Wang et al., 2024). Both amino acids were involved in the protein metabolism of tissues. Proline reflected the extracellular matrix turnover and asparagine could be de novo synthesised from proline. The onset of lactation was promoting not only lipolysis but also protein turnover in both breeds, however, more pronounced in HF cows. The reduction in BW of HF cows reflected that difference.

Alpha amino adipic acid is an oxidation product of lysine and used as a marker for oxidative stress in human medicine (Estaras et al., 2020). In dairy cows, early lactation and a high milk yield have been shown to be accompanied by enhanced oxidative stress (Hanschke et al., 2016). Thus, the higher concentrations of α-AAA in HF cows at day +21 might reflect a higher oxidative burden due to higher milk yield and body mass mobilisation in this breed at that time point. In human medicine, α-AAA is an early marker for diabetes risk (Wang et al., 2013) and has various impacts on glycolipid metabolism and insulin secretion (Xu et al., 2019). An inverse relationship could also be suggested for dairy cows. Lower insulin concentrations in plasma and thereby, a higher oxidative stress level at the onset of lactation could have caused an imbalanced condition in HF cows resulting in higher body mass loss, most likely due to lipid and protein mobilisation for producing milk, although these conditions led to a higher milk yield. However, causes and consequences are difficult to identify from this experimental setting. An important indicator of balancing oxidative stress is kynurenine, a reactive oxygen species scavenger, which concentrations were strongly increased at the onset of lactation only in HF cows. Kynurenine is known to have anti-oxidant, anti-inflammatory and neuroprotective effects (Ramirez Ortega et al., 2022). Thus, in HF cows, higher kynurenine concentration at the onset of lactation might reflect stimulation of a pathway, the well-described kynurenine pathway (Esquivel Gonzalez et al., 2017), to ameliorate and balance cellular oxidative stress and metabolic inflammation.

Biogenic amines are commonly attributed to exert anti-oxidative, anti-inflammatory effects (Matsumoto et al., 2011). In this study, the biogenic amine sarcosine expressed lower concentrations at the onset of lactation in HF cows while SI cows had constant sarcosine concentrations. This reduction of sarcosine was also found in muscle and serum of HF cows in transition from 1 week before to 1 week after parturition (Sadri et al., 2024). A decrease in sarcosine might announce a weakness in cellular proteostasis (Walters et al., 2018) supporting further the idea that HF cows of the present study were in a more catabolic situation compared to SI cows at the onset of lactation. Furthermore, in dairy cows, that left productive life within the current lactation due to health and fertility disorders, lower concentrations of sarcosine were found in comparison to their herd mates that remained in the herd (Huber et al., 2016). Zheng et al. (2016) found lower concentrations of sarcosine in mid–lactation dairy cows affected with footrot compared to non-affected cows and attributed this finding to higher protein utilisation in that group. To sum up, this biogenic amine is an important metabolic signal and appears to indicate breed differences, at least between HF and SI cows.

Further substrate classes

Acylcarnitines, phosphatidylcholines, lysophosphatidylcholines and sphingomyelines were only marginally influenced by time and breed. All substrate classes are not well studied in farm animals such as dairy cows. Thus, it is difficult to speculate about the biological meaning of changes over time and differences between breeds. For glutaconylcarnitine (C5:1 DC), a slight breed effect was detected with higher values in SI cows after the onset of lactation. Glutaconylcarnitine was described as a leptin-associated metabolite in humans, indicating greater weight gains with higher plasma glutaconylcarnitine concentrations (Perng et al., 2016). This short-chain acylcarnitine could indicate a more balanced metabolism in SI cows without losing their BW.

Conclusions

Classical and upcoming novel metabolic indicators like BCAA, α-AAA and kynurenine strongly reflected that HF cows have a more tensed metabolic condition at the onset of lactation than SI cows and therefore, develop signs of an enhanced catabolic state, higher oxidative stress and less efficient NEFA utilisation. Whether the 15–20% higher milk yield of HF cows were responsible for this metabolic condition remained unclear. However, in this study, HF cows appeared to allocate their resources into milk and less into body mass. SI cows expressed a more stable and less pro-oxidative metabolic condition with constant body mass but lower milk yield, indicating that they were able to manage their resource allocation until day 21 pp more physiologically. This was potentially the result of a genetically based decision for resource allocation in each breed. Further studies are needed to identify traits for the resource allocation pathways. Especially, the meaning of the systemic protein turnover appears to be of interest since BCAA are indicators of skeletal muscle degradation which is often associated with peripheral insulin insensitivity. Whether insulin insensitivity is the cause or the consequence of muscle degradation is still an open question, also in human metabolic research.

Ethics approval

All procedures involving animals in the present experiment were performed according to the German animal welfare law and were approved by the Government of Upper Bavaria under the project number 55.2-1-54-2532.0-66-2016.

Data and model availability statement

None of the data were deposited in an official depository. However, a full set of metabolomics data are available upon request (korinna.huber@uni-hohenheim.de).

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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