PAPER

Association of index of welfare and metabolism with the genetic merit of Holstein and Simmental cows after the peak of lactation

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Abstract

The study investigated the relationship of markers of welfare and metabolism in milk, urine and blood with the genetic merit of Holstein and Simmental cows after the peak of lactation. Cows were selected from 3 Simmental (IS) and 2 Holstein (IH) commercial dairy farms. Within each farm, cows were ranked according to the estimated breeding value for milk protein yield (EBVp) from minus to positive and selected every 5 EBVps from minus to positive values (about 20% lactating cows for each farm). Milk was sampled and analysed for protein, fat, lactose, cortisol contents and somatic cell count (SCC). Blood and urines were analysed for biomarkers of metabolism and welfare. Significantly lower body condition score (BCS) was observed for IH in comparison to IS. Plasma creatinine was higher in IS, whilst Zn, total antioxidant status and glutathione peroxidase was higher in IH. The creatinine N to N ratio in urine was significantly higher for IS, while the purine derivatives (PD) N to creatinine N ratio was higher for IH. The EBVp was negatively related to BCS and glucose for IS and to plasma β-hydroxybutyrate in both breeds. EBVp was negatively related to urinary PD N to total N ratios for IS and to PD N to creatinine N ratio for IH. These preliminary results would indicate that the selection of cows for milk protein yield had minor effect on plasma and milk biomarkers of welfare. Instead, biomarkers of metabolism were more affected by breed and genetic merit.

Introduction

The genetic merit for milk yield and composition of dairy cows is estimated from phenotypic data registered in the farms during the official records and with the application of appropriated statistical models (Pritchard *et al.*, 2013). Before the advent of genomic selection, the genetic progress of animals was largely dependent from the results derived from the statistical models elaborated by quantitative genetic, which define the estimated breeding values (EBV) for each of the recorded traits (Hayes *et al.*, 2009). The continuous selection based on the EBV and the collaboration among Countries has led to the progress of the productive traits, as milk yield and its constituents. In the high yielding dairy cows, a deterioration in reproductive performance and higher disease susceptibility has been observed and this is considered a negative consequence of the selection programs that had as priority only productive traits (Berry, 2011; Oltenacu and Broom, 2010; Snijders *et al.*, 2001).

The associated variations of metabolic efficiencies and welfare of the dairy cows under selective pressure is a fascinating field of research (Kelly *et al.*, 2011; Cassandro *et al.*, 2013) that requires further investigations. Several studies on animal welfare are currently ongoing to gather information to readdress breeding programs in order to recover the health and reproductive performances of cows.

The assessment of digestive and metabolic efficiencies is very complex, considering the countless physiological factors affecting these processes, and biomarkers can represent an alternative approach to investigate these aspects. Non-esterified fatty acids (NEFA) concentration, β-hydroxybutyrate (BHB) and urea in blood, BHB and urea in milk (Kelly *et al.*, 2010), total purine derivatives (PD) excretion (Stefanon *et al.*, 2001) or PD to creatinine ratios (Susmel *et al.*, 1995) in blood and urine have been already used as biomarkers of metabolism and feeding efficiency. Variations of blood metabolites are not only related to feed intake, diet composition, and physical activity, but also depend from the genotype of the animals (Herd and Arthur, 2009; Penasa *et al.*, 2014). Recent researches (Karisa *et al.*, 2014; Kelly *et al.*, 2011) examined these processes in productive cattle, providing evidence of association between mitochondrial biogenesis and energetic efficiency and suggesting that the expression of some genes and their products may provide potential indicators for genetic variation of feed efficiency.

The aim of this study was to examine the effect of EBV for milk protein (EBVp) and breed on haematological, milk and urine metabolites in lactating cows. We hypothesized that different breed and genetic merit for milk protein, as reflected by the EBVp of the animals, can impact markers of metabolism and welfare in biological fluids. The study was conducted in commercial farms of Italian Simmental and Italian Holstein cows after the peak of lactation, to avoid metabolic imbalance related to the onset of lactation.

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Key words: Biomarkers; Metabolism; Welfare; Genetic merit; Lactating cow.

Funding: the research was realized within the Project BELLIMPRESA/ Rationalizations of internal resources and dissemination of a business culture aimed at the multi-functionality and economic sustainability of farms, funded in the context of the Cross-Border Cooperation Program Italy-Slovenia 2007-2013 by the European Fund of regional development and by national funds.

Received for publication: 12 January 2015.
Accepted for publication: 31 March 2015.

Italian Journal of Animal Science 2015; 14:3841
doi:10.4081/ijas.2015.3841

Materials and methods

Animals

One hundred fifty three lactating cows were selected from 3 Simmental and 2 Holstein commercial dairy farms located in Italy, Friuli Venezia Giulia Region. Farms were sorted for having homogeneous management and diet compositions before and during the experimental period. The local Breeder Association (Associazione Allevatori del Friuli Venezia Giulia, Codroipo, UD, Italy) provided assistance for farm selection and information about individual milk records through the lactation, reproductive parameters and managerial aspects. The Italian Holstein (IH) and Italian Simmental (IS) breeder associations provided updated EBV for milk protein content (EBVp) of the cows involved in the study. Lactating cows with days in milk (DIM) ranging from 70 to 250 days, clinically healthy and with parity

from 2 to 6 (mean 3.0±1.1) were identified. Within each farm, cows were first ordered according to the EBVp from minus to positive values and one animal every 5 EBVp values was selected for the study (about 20% lactating cows for each farm). After this selection parity ranged from 2 to 4. All procedures were performed in respect of the Italian legislation on animal care (D.L. n. 116, 27/1/1992) and the internal rules of University of Udine.

Data collection

The lactating cows, allotted to the same box, were fed *ad libitum* a total mixed ration (TMR) offered twice a day, after the morning and the afternoon milking. Starting from 1 week before the day of sampling, the composition of the rations and the amounts of TMR offered were recorded from the register of the TMR mixed feeder. Samples of TMR were collected the day of sampling from the manger and were analysed for dry matter (DM; 105°C for 12 h), ash (512°C for 8 h), crude protein (CP), ether extract (EE), neutral detergent fibre (NDF) and starch with standard methods (AOAC, 2012). The net energy of lactation and the digestible protein in the intestine (PDIE, PDIN) were calculated from the chemical data and from data reported in tables of INRA (1989). The 5 farms were sampled in the period from April to May. The day of official milk recording made by the Breeder Association, 100 ml of milk samples were collected from each cow at the morning milking. An aliquot of 50 ml of milk was transferred into a tube containing preservative and was used for protein, fat, lactose analyses and for somatic cell count (SCC) determination. A second aliquot of 50 mL of milk was collected without preservative, frozen within 2 h and stored at -20°C for BHB and cortisol analyses. After milking and before the morning meal, when cows had *ad libitum* access to fresh water and spontaneously moved to cattle feed headlock fence, blood was sampled from the coccygeal vein in 10 ml vacuum tubes with Li-heparin and K3-EDTA (Venoject, Terumo Europe N.V., Leuven, Belgium). Blood was centrifuged within 1 h at 3000 RPM for 10 min at 20°C and plasma samples were stored at –20°C until further analyses. Urine was sampled after stimulation of micturition. Ten ml of sample was immediately added with 10% sulphuric acid until a final pH of 3.0 was reached and the amount of acid added was recorded. The samples were filtered using a 0.22 m membrane filter (Millipore Corporation, Billerica, MA, USA) and 3 aliquots of each sample were stored at -20°C until analysis. The day of sampling, the body condition score (BCS) of each cow was recorded by the same experienced observer on a scale from 1 (thin) to 5 (fat) with 0.25 point intervals (Edmonson *et al*., 1989).

Analysis of biological fluids

Total protein, albumin, urea, glucose and creatinine were analysed using a Roche Cobas® 6000 analyser with proprietary kits (TP2, ALB2, GLUC3, UREAL and CREP2; F. Hoffmann-La Roche AG, Basel Switzerland). NEFA, BHB, glutathione peroxidase (GPx) and total antioxidant status (TAS) were measured with Randox kits (FA 115, RB1008, RS504, NX2332; Randox Laboratories Limited, Crumlin, UK). GPx was expressed as units of Hb. Plasma Zn was analysed with the Sentinel kit (17640H, Sentinel CH SpA, Milano, Italy). Milk protein, fat, lactose contents and SCC were analyzed with mid infrared spectroscopy (MIR, Fourier Transform Instrument, FT6000, Foss Electric, Hillerød, Denmark).

Urine samples were analysed for total N with Kjeldahl method, creatinine with Jaffe method (Hawk *et al.*, 1976) and urea with Berthelot method (Randox kit UR 1068, Randox Laboratories Limited, Crumlin, UK). Uric acid and allantoin were measured using the HPLC method (Piani *et al.*, 2004). Purine derivative (PD) N was calculated as the sum of allantoin N and uric acid N.

Cortisol assay in milk and in plasma

Milk cortisol was analysed in skimmed milk, after centrifugation (1500 g, 4°C, 15 min). Plasma samples (0.1 mL) were extracted with 8 ml diethyl ether. The ether fractions were transferred into fresh glass tubes and dried under nitrogen. The dry extracts were carefully dissolved in 0.2 ml assay buffer. Skim milk and plasma extracts were assayed by a solid-phase microtitre RIA (Gabai *et al.*, 2006), using a Microscint 20 instrument (Perkin-Elmer Life Sciences, Monza, MB, Italy) and counted on the beta-counter (Top-Count; Perkin-Elmer Life Sciences). All samples were assayed in duplicate. The sensitivity of the assay was defined as the dose of hormone at 90% binding (B/B0) and was 3.125 pg/well. The intra-assay and inter-assay coefficients of variation in high and low cortisol pooled plasma samples were 5.9%, 9.1%, 13.5%, and 15.1%, respectively.

Statistical analysis

All the data, referring to a single sample for each cow, were stored in a spreadsheet using Microsoft Office Excel (2010, Microsoft Corp., Redmond, WA, USA) and statistical analyses were performed with the SPSS package (1997). Normality of data was tested by the Kolmogorov-Smirnov non parametric test. Only SCC was not normal distributed and a log(2) transformation was used before statistical analysis. A mixed procedure was used for the outcomes of milk yield and its composition, BCS, blood and urine metabolites according to the following model:

$$y_{ijk} = \mu + B_i + F(B)_{ij} + EBVp(B)_i + DIM + \varepsilon_{ijk}$$

where
y_{ijk} is the dependent variable (milk yield and its composition, BCS, blood and urine metabolites);
μ is the general mean; B_i is the fixed effect of the *i*th breed (i = 1-2);
$F(B)_{ij}$ is the random effect of the *j*th farm (j =1-5) nested within the *i*th breed (i =1-2);
$EBVp(B)_i$ is the covariate for EBVp nested within the *i*th breed (i = 1-2);
DIM is the covariate for days in milk;
ε_{ijk} is the random residual.
All tests were 2-tailed and significance was based on a P<0.05.

Results

The characteristics of the farms involved in the study and the composition of the herds are reported in Table 1 and the ingredients of the rations for the 5 farms in Table 2. The effects of breed, farm within breed and the covariates for DIM and EBVp within breed are reported in Table 3 for BCS, milk yield and its composition. For the IH cows, significantly higher milk (P<0.001) and fat (P<0.01) yields and significantly lower values (P<0.001) of BCS, protein percentage, SCC and urea were observed in comparison to IS. The linear effect of DIM was positive for BCS (P<0.05), milk protein percentage and SCC (P<0.01) and negative for milk yield (P<0.01), milk fat and protein yields (P<0.05). A positive linear effect of EBVp was observed for milk and protein yields (P<0.01), fat yield (P<0.05) and milk protein percentage (P<0.01) for IH cows. For the IS cows, the EBVp was inversely related with BCS (P<0.05) and was positively related to milk yield (P<0.01), milk fat yield (P<0.05) and protein yield (P<0.01). A significant effect of farm, but not breed and EBVp, was observed for milk (P<0.05) and plasma (P<0.01; Table 4) cortisol.

The effects of breed, farm within breed and the covariates for DIM and EBVp within breed for haematological variables are reported in Table 4. Higher mean values for creatinine (P<0.001) and lower mean values for Zn, TAS and GPx (P<0.001) were observed in IS in

comparison to IH cows. The DIM was linearly related only to plasma urea (P<0.05). The EBVp was negatively related to glucose in the IS cows (P<0.05) and to BHB in both breeds (P<0.05). The concentrations of N, creatinine and the creatinine to N ratio in urine samples (Table 5) were significantly higher (P<0.01) in IS than IH cows. The PD N to total N ratio (P<0.05) and PD N to creatinine N ratio (P<0.001) were lower in IS than in IH cows. The DIM linearly affected the creatinine to total N ratio (P<0.05). EBVp was negatively related to the ratio between urea and total N and the ratio between PD N to total N (P<0.05) in IS cows, and the ratio between PD N and creatinine N (P<0.05) in IH cows.

Discussion

This study investigated whether differences in genetic merit and breed of cows after peak of lactation can impact on metabolism and welfare as assessed with biomarkers in milk, blood and urine. Among the EBVs, we concentrated our attention to the milk protein yield, as this trait is the combination of protein percentage and milk yield. Productive performances at the time of sampling were related to EBVp and breed (Table 3), confirming that the selection criteria of the cows, based on estimated performance, reflects the differences of their genetic background.

A high positive genetic correlation between milk and protein yield has been reported (Lipkin *et al.*, 2008), while for milk fat and protein percentage the correlation with milk production is negative (Viitala *et al.*, 2003). At the same time the lack of QTL affecting only protein yield was confirmed by both these studies and Lipkin *et al.* (2008) in Israeli Holstein cows reported that 68.9 and 76.5% of QTL markers affecting protein yield were also associated to protein percentage and milk yield, respectively. The IS is a dual purpose breed and the selection combines milk and meat production traits (www.anapri.it) with a breeding scheme differing from that of IH breed, which does not consider meat traits (www.anafi.it) and for this reason a linear regression of EBVp within breed was used in the statistical model.

The biomarkers measured in the present study are often used for diagnostic purposes or to verify the health conditions or the response of animals to specific treatments. Furthermore, biomarkers are often measured during the peripartum, when cow's response is largely affected by environmental conditions, as BCS, diet composition and feeding regimes, calving, management system and milking hygiene (Stefanon *et al.*, 2005; Graugnard *et al.*, 2012). Fewer information is given about the relationship of cow genetic background with biomarkers of welfare and metabolism measured in blood, urine and milk in mid lactating cows. Our hypothesis is that environmental effects are minimized after the transition period, when cows enter into a more sta-

Table 1. Composition of the herds and characteristics of the farms involved in the study.

		Farm				
		A	B	C	D	E
Breed		IS	IS	IS	IH	IH
Herd size	n	343	270	216	368	433
Dairy animals	n	183	169	119	194	215
Heifers	n	65	61	43	76	97
Lactating cows	n	152	148	99	155	182
Cows <70 DIM	n	31	16	12	14	22
Cows sampled	n	27	33	20	36	39
	%	22.3	25.6	23.0	25.5	24.4
DIM	Mean	126.7	141.4	141.8	151.0	145.8
EBVp	Mean	17.9	8.6	-1.2	22.6	16.5
Housing	Type	Free stall	Free stall	Free stall	Free stall	Free stall
Bedding	Type	Concrete	Straw	Concrete	Concrete	Concrete
Milking	Type	Parlour	Parlour	Parlour	Parlour	Parlour

IS, Italian Simmental; IH, Italian Holstein; DIM, days in milk; EBVp, estimated breeding values for protein.

Table 2. Composition of the rations offered to the dairy cows and their chemical and nutritive contents.

	Farm				
	A	B	C	D	E
Ingredients, kg DM/d					
Lucerne, hay	3.06	4.45	3.13	2.50	4.03
Grass, hay					0.90
Corn, silage	6.82	6.06	6.00	7.82	6.15
Corn cob, silage	3.13	3.24	3.54		
Lucerne, silage	1.50		3.00	3.16	
Grass, silage			0.71		
Corn, ground	0.87	1.04	0.88	3.15	4.56
Soybean meal	1.05	0.70	2.19		1.75
Rapeseed meal					0.90
Whole Soybean	1.25				
Barley, ground		0.44			
Wheat bran				0.88	
Protein and fat supplements	2.38	2.64	0.45	3.17	2.45
Minerals	0.20	0.05	0.10	0.55	0.05
Total	20.3	19.3	20.2	20.3	20.8
Composition, %DM					
Crude protein	15.6	15.1	15.7	14.7	15.4
Ether extract	4.0	2.4	3.0	3.3	4.2
Ash	7.6	6.3	5.9	6.8	5.4
Neutral detergent fibre	31.9	33.9	34.4	34.3	32.5
Starch	26.6	27.9	25.3	25.8	27.9
PDI, g/d	2077	1963	2109	1604	1954
PDIE	2024	1804	1921	1526	1784
NEl, MJ/d	125.7	114.6	119.2	114.1	120.2

DM, dry matter; PDI, protein digested in the small intestine; PDIN, amount of protein digested in the small intestine produced from rumen undegraded dietary protein and by microbial protein when the supply of rumen degradable N is limited (INRA, 1989); PDIE, amount of protein digested in the small intestine produced from rumen undegraded dietary protein and by microbial protein when the supply of rumen fermentable organic matter is limited (INRA, 1989); NEl, net energy for lactation.

bilized phase of lactation.The significantly higher SCC in IS in comparison to IH cows could be related to the different breeding scheme applied in Italy, since the weight of SCC trait accounts for 5% in IS and 10% in IH (www.anapri.it; www.anafi.it). Penasa *et al*. (2014), comparing milk data collected from Brown Swiss, Holstein Frisian and Simmental cows, reported a significantly lower SCC score for the latter breed in comparison to the other two. The study refers to a larger dataset of animals and to 39 commercial multibreed farms and the differences with our results can be due to the limited number of animals considered in the present study. Moreover, in our conditions, differences in management and environment of farms can have affected the SCC in milk more than genetic background. However, in the light of the results of other biomarkers (Table 4), a more favourable immune surveillance in IH cows can be supposed. The significantly lower plasma Zn of IS cows in comparison to IH cows would support a healthier condition of the latter animals. Administration of dietary Zn has been reported to reduce milk SCC (Sobhanirad *et al.*, 2010), since this mineral improves immune function by activating cell-mediated immune responsiveness and plays a role in keratin formation of the teat canal. Furthermore, blood GPx, an antioxidant enzyme inversely related to oxidative stress in dairy cows (Stefanon *et al.*, 2005) and TAS, which expresses the total antioxidant capacity of plasma, would also support a lower involvement of inflammatory cascade in IH cows. It is well known that an important component of the immune response is oxidative burst, during which superoxide anion radicals are produced from oxygen, and consequently cause a perturbation in the oxidative balance of the animal. Again, if this is more related to genetic bases or to environmental conditions of farms deserves further investigation.

Under stressful conditions the hypothalamic-pituitary-adrenal (HPA) axis, the autonomic nervous system, and the immune system are recruited to re-establish homeostasis (Colitti *et al.*, 2007; Amadori *et al.*, 2009; Sgorlon *et al.*, 2012). Cortisol is a gold standard to measure HPA stimulation, but its concentration in blood is affected by sampling technique and sudden environmental modifications, leading to a pulsatile secretion of this biomarker. In our study the correlation between plasma and milk cortisol was not significant (data not shown). Milk can be proposed as an alternative sampling site for cortisol determination, since it does not require manipulation of animals, better reflecting the response to environment of cows (Fukasawa and Tsukada, 2010). Even though breed and EBVp did not affect milk cortisol, the variation between farms indicates that this measure is promising to monitor the influence of environmental conditions in cows. However, the understanding of how individual differences are affected by genetics requires further investigations (Gygax *et al.*, 2006).

The BHB and NEFA contents in plasma are reliable markers of energy metabolism at the beginning of lactation (McArt *et al.*, 2013), when a large mobilization of fat stored in the tissues in high yielding cows occurs, but less information is reported in the later phase of lactation, when the recovery of DMI allows to cover the energy requirements for maintenance and for milk production. A significant and negative linear relation was found between BHB in plasma and EBVp for IS and IH cows (Table 4). It is likely that cows with higher genetic merit can use energy more efficiently. BHB concentration in plasma is mainly used to diagnose sub clinical ketosis during the peripartum (Duffield, 2000), but evidences in growing cattle suggest that it can also be considered a marker of metabolic efficiency and residual feed intake (Kelly *et al.*, 2010). Moreover, BHB can reduce feed intake and depress pituitary and thyroid functions, which are both strongly implicated in homeostatic control (Laeger *et al.*, 2010).

Complete nitrogen balance technique is used to assess nitrogen efficiency for lactation, but total urine collection is not feasible in trials involving cows in commercial farms. Alternatively, the concentration of N and of nitrogenous compounds can be used as an indicator of whole body and rumen nitrogen metabolism and usage (Gruber *et al.*, 1999). The significantly higher urinary total N content in IS in comparison to IH cows is related to the higher creatinine N concentration of the dual purposes cows (Table 5). Moreover, the significantly higher urine concentrations of creatinine in IS cows corresponds to a higher plasma creatinine concentration (Table 4) and is probably related to the body composition of the dual purpose breed in comparison to IH. As a product of muscle metabolism, creatinine excretion has been directly related to muscle mass, as diet composition has a relatively minor effect on creatinine excretion (Chen *et al.*, 1995). As reported from the IS breeder association (www.anapri.it), the muscularity accounts for 6% in the selection scheme whilst

Table 3. Effects of breed, days in milk and genetic merit on body condition score, milk yield and its composition, somatic cell count, urea and cortisol contents in Italian Holstein and Italian Simmental lactating cows, sampled in 5 commercial farms in North-East of Italy.

	Breeds		SEM	Effects				
	IH	IS		Breed	Farm	Covariates		
						DIM	EBVpIH	EBVpIS
BCS	2.75	3.14	0.03	***	***	* (+)	ns (-)	* (-)
Milk output, kg/d	35.34	30.98	4.98	***	***	** (-)	** (+)	** (+)
Milk								
Fat	1.33	1.16	0.02	**	***	* (-)	* (+)	* (+)
Protein	1.10	1.08	0.02	ns	***	* (-)	** (+)	** (+)
Milk composition, %								
Fat	3.79	3.78	0.06	ns	*	ns (+)	ns (+)	ns (-)
Protein	3.12	3.49	0.02	***	***	** (+)	** (+)	ns (+)
SCC, count	4.22	5.05	0.13	***	***	** (+)	ns (+)	ns (-)
Urea, mmol/L	17.10	23.30	3.67	***	***	ns (-)	ns (-)	ns (+)
Cortisol, pmol/L	1.01	0.88	0.04	ns	*	ns (-)	ns (+)	ns (+)

IH, Italian Holstein; IS, Italian Simmental; DIM, days in milk; EBVpIH, estimated breeding values for protein of Italian Holstein cows; EBVpIS, estimated breeding values for protein of Italian Simmental cows; BCS, body condition score; SCC, somatic cell count; *P<0.05; **P<0.01; ***P<0.001; ns, not significant; (+), positive covariates for the variable; (-), negative covariates for the variable.

Table 4. Effects of breed, days in milk and genetic merit on plasma and blood parameters in Italian Holstein and Italian Simmental lactating cows, sampled in 5 commercial farms in North-East of Italy.

	Breeds		SEM	Effects				
	IH	IS		Breed	Farm	Covariates		
						DIM	EBVpIH	EBVpIS
Total protein, g/L	83.6	80.6	6.0	ns	***	ns (-)	ns (+)	ns (+)
Albumin, g/L	37.5	37.2	2.5	ns	***	ns (-)	ns (+)	ns (+)
Urea, mmol/l	4.8	5.0	0.1	ns	***	* (-)	ns (-)	ns (-)
Creatinine, µmol/l	63.2	90.0	8.3	***	***	ns (+)	ns (+)	ns (-)
Zinc, µmol/l	12.9	11.2	0.2	***	**	ns (+)	ns (-)	ns (+)
TAS, mmol/l	1.2	1.1	0.1	***	ns	ns (+)	ns (+)	ns (+)
GPx, U/gHb	326	243	52	***	***	ns (+)	ns (-)	ns (+)
Cortisol, pmol/ml	9.6	8.7	0.6	ns	***	ns (+)	ns (-)	ns (+
Glucose, mmol/l	3.40	3.40	0.02	ns	***	ns (+)	ns (+)	* (-)
NEFA, meq/l	0.12	0.13	0.01	ns	**	ns (+)	ns (+)	ns (-)
BHB, mmol/l	0.58	0.55	0.01	ns	***	ns (+)	* (-)	* (-)

IH, Italian Holstein; IS, Italian Simmental; DIM, days in milk; EBVpIH, estimated breeding values for protein of Italian Holstein cows; EBVpIS, estimated breeding values for protein of Italian Simmental cows; TAS, total antioxidant status; GPx, glutathione peroxidase; NEFA, non-esterified fatty acids; BHB, β-hydroxybutirate. *P<0.05; **P<0.01; ***P<0.001; ns, not significant; (+), positive covariates for the variable; (-), negative covariates for the variable.

Table 5. Effects of breed, days in milk and genetic merit on urine parameters in Italian Holstein and Italian Simmental lactating cows, sampled in 5 commercial farms in North-East of Italy.

	Breeds		SEM	Effects				
	IH	IS		Breed	Farm	Covariates		
						DIM	EBVpIH	EBVpIS
Nitrogen fractions, g/L								
Total N	6.52	7.94	3.54	**	***	ns (-)	ns (-)	ns (+)
Urea N	3.96	3.44	4.30	ns	ns	ns (-)	ns (-)	ns (-)
Creatinine N	0.90	1.35	0.03	***	**	ns (+)	ns (+)	ns (+)
PD N	0.87	0.83	0.02	ns	ns	ns (+)	ns (+)	ns (-)
Ratios, unit								
Creatinine N:total N	0.15	0.17	0.01	***	***	* (+)	ns (+)	ns (-)
Urea N:total N	0.65	0.45	0.03	ns	***	ns (+)	ns (-)	* (-)
PD N:total N	0.14	0.11	0.01	*	***	ns (+)	ns (+)	* (-)
PD N:creatinine N	0.97	0.61	0.06	***	ns	ns (-)	* (-)	ns (-)

IH, Italian Holstein; IS, Italian Simmental; DIM, days in milk; EBVpIH, estimated breeding values for protein of Italian Holstein cows; EBVpIS, estimated breeding values for protein of Italian Simmental cows; N, nitrogen; PD, purine derivatives; P<0.05; **P<0.01; ***P<0.001; ns, not significant; (+), positive covariates for the variable; (-), negative covariates for the variable.

is not considered for IH cows.

Urinary excretion of PD N has been proposed as a marker of rumen microbial protein supply (Stefanon *et al.*, 2001), but also this technique requires total daily urine collection. Alternatively, PD N to creatinine N ratio can be used in a spot sample (Chen *et al*., 1995). The significantly higher PD N to creatinine N ratio measured in urine samples of IH cows (Table 5) support a higher microbial protein supply for this breed. Furthermore, the negative effect of EBVp observed for urea N to total N ratio and for PD N to total N ratio in IS and for PD N to creatinine N ratio in IH could indicate a more efficient nitrogen utilization in the cows with higher genetic merit.

Conclusions

Although this study refers to a restricted number of animals, these preliminary results suggest that selecting dairy cows for higher milk protein yield has minor impact on biomarkers of welfare. Instead, biomarkers of energy and protein metabolism were more influenced by breed and genetic selection. From the results, definitive considerations cannot be drawn and further studies are needed to ascertain the relationship between genetic components and welfare in dairy cows. However the approach of the present study can help to understand which can be the effect of the selection on metabolism and welfare conditions of dairy cows.

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