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 R 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; Otenacu 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.

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) breeders 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

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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, 27/1992) 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 vacu-umb 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 Rohe AG, Basel Switzerland). NEFA, BHB, glutathione peroxidase (GPx) and total antioxidant status (TAS) were measured with Random kits (FA 115, RB1008, RS504, NX2332; Random 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 analysed 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 (Random kit UR 1068, Randomo 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 microtiter RIA (Gabi et al., 2006), using a Microcist 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%, respective-ly. 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 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.

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:

\[ y_{ijk} = \mu + B_i + F(B)_{ij} + EBVp(B)_i + DIM + \epsilon_{ijk} \]

where \( y_{ijk} \) is the dependent variable (milk yield and its composition, BCS, blood and urine metabolites);
\( \mu \) 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;\( \epsilon_{ijk} \) is the random residual.

All tests were 2-tailed and significance was based on a P<0.05.
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 in 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 (P<0.05). 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 (Viitola 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.anapr.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. |
|-----------------------------------------|--------|--------|--------|--------|--------|
| 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     |
| 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. |
|-----------------------------------------|--------|--------|--------|--------|--------|
| 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                               | 2877   | 1963   | 2109   | 1604   | 1954   |
| PDIE                                   | 2024   | 1804   | 1921   | 1526   | 1784   |
| NEI, M/d                               | 125.7  | 114.6  | 119.2  | 114.1  | 120.2  |

IS, dry matter; PDI, protein digested in the small intestine; PDIN, amount of protein digested in the small intestine 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 from rumen undegraded dietary protein and by microbial protein when the supply of rumen fermentable organic matter is limited (INRA, 1989); NEI, net energy for lactation.
The significantly higher SCC in IS in comparison to IH cows is related to 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 musculature 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 | Breed | Farm | Effects |
|--------|-----|-------|------|---------|
| IH | IS | | | 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 (+) | ns (+) |
| SCC, count | 4.22 | 5.05 | 0.13 | *** | *** | ** (+) | ns (+) | ns (-) |
| Urea, mmol/L | 17.10 | 23.30 | 3.67 | *** | *** | * (-) | 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<0.01; **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 | Covariates |
|--------|-----|---------|------------|
| IH     | IS  | Breed   | Farm       | 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 (+) | 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 (+) | ns (+) | ns (-) |

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 | Covariates |
|--------|-----|---------|------------|
| IH     | IS  | Breed   | Farm       | DIM | EBVpIH | EBVpIS |
| Nitrogen fractions, g/L | | | | | | |
| Total N | 6.52 | 7.94 | 3.54 | ** | *** | ns (-) | ns (-) | ns (+) |
| Urea N  | 3.86 | 3.44 | 4.30 | ns | ns | ns (-) | ns (-) | ns (-) |
| Creatinine N  | 0.90 | 1.35 | 0.03 | *** | ** | ns (+) | ns (+) | ns (+) |
| PD N    | 0.67 | 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 (-) |

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.

References

Amadori, M., Stefanon, B., Sgorlon, S., Farinacci, M., 2009. Immune system response to stress factors. Ital. J. Anim. Sci. 8:287-299.

AOAC. 2012. Official methods of analysis, 19th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Berry, D., Bermingham, M.L., Good, M., More, S.J., 2011. Genetics of animal health and disease in cattle. Irish Vet. J. 64:5.

Cassandro, M., Mele, M., Stefanon, B., 2013. Genetic aspects of enteric methane emission in livestock ruminants. Ital. J. Anim. Sci. 12:e73.

Chen, X.B., Susmel, P., Stefanon, B., Orskov, S., 2001. A new method for the determination of urea and glucose. J. AOAC Int. 84:456-460.

Sgorlon, S., Chen, X.B., Susmel, P., Stefanon, B., Sgorlon, S., et al., 2015. Genetic and nongenetic aspects of milk protein yield and metabolites in Italian Holstein and Italian Simmental dairy cows. Ital. J. Anim. Sci. 14:2015.

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.
E.R., 1995. On the use of purine derivatives in spot urine, plasma and milk samples as indicators of microbial protein supply in sheep and cattle. pp 325-329 in Proc. 7th Int. Symp. Protein Metab. Nutr., Santarem, Portugal.

Colitti, M., Sgorlon, S., Stradaioi, G., Farinacci, M., Gabai, G., Stefanon, B., 2007. Grape polyphenols affect mRNA expression of PGHS-2, TTS11b and FOXO3 in endometrium of heifers under ACTH-induced stress. Theriogenology 68:1022-1030.

Duffield, T., 2000. Subclinical ketosis in lactating dairy cattle. Vet. Clin. North Am. Food Anim. Pract. 16:231-253.

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68-78.

Fukasawa, M., Tsukada, H., 2010. Relationship between milk cortisol concentration and the behavioral characteristics of postpartum cows introduced to a new group. Anim. Sci. J. 81:612-617.

Gabai, G., Mollo, A., Marinelli, L., Badan, M., Bono, G., 2006. Endocrine and ovarian responses to prolonged adrenal stimulation at the time of induced CL regression. Reprod. Domest. Anim. 41:485-493.

Graugnard, D.E., Bionaz, M., Trevisi, E., Moyes, K.M., Salak-Johnson, J.L., Wallace, R.L., Drackley, J.K., Bertoni, G., Loor, J.J., 2010. Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum. J. Dairy Sci. 95:1749-1758.

Gruber, L., Steinwidder, A., Stefanon, B., Steiner, B., Steinwender, R. 1999. Influence of grassland management in Alpine regions and concentrate level on N excretion and milk yield of dairy cows. Livest. Prod. Sci. 61:155-170.

Gygax, L., Neuffer, I., Kaufmann, C., Hauser, B.J., Bowman, P.J., Chamberlain, A.J., Goddard, M.E., 2009. Invited review: genomic selection in dairy cattle: progress and challenges. J. Dairy Sci. 92:433-443.

Her, R.M., Arthur, P.F., 2009. Physiological basis for residual feed intake. J. Anim. Sci. 87(14 Suppl.):E64-E71.

INRA, 1988. Alimentation des bovins, ovins et caprins. Institut Nationale de la Recherche Agronomique ed., Paris, France.

Karisa, B., Moore, S., Plastow, G., 2014. Analysis of biological networks and biological pathways associated with residual feed intake in beef cattle. Anim. Sci. J. 85:374-387.

Kelly, A.K., McGee, M., Crews, D.H. Jr, Sweeten, T., Boland, T.M., Kenny, D.A., 2010. Repeatability of feed efficiency, carcase ultrasound, feeding behavior, and blood metabolic variables in finishing heifers divergently selected for residual feed intake. J. Anim. Sci. 88:3214-3225.

Kelly, A.K., Waters, S.M., McGee, M., Fonseca, R.G., Carberry, C., Kenny, D.A., 2011. mRNA expression of genes regulating oxidative phosphorylation in the muscle of beef cattle divergently ranked on residual feed intake. Physiol. Genomics 43:12-23.

Laeger, T., Metges, C.C., Kuhla, B., 2010. Role of b-hydroxybutyric acid in the central regulation of energy balance. Appetite 54:450-455.

Lipkin, E., Tal-Stein, R., Friedmann, A., Soller, M., 2008. Effect of quantitative trait loci for milk protein yield and milk yield in Israeli Holstein dairy cattle. J. Dairy Sci. 91:1614-1627.

McArt, J.A.A., Nydam, D.V., Oetzel, G.R., Overton, T.R., Ospina, P.A., 2013. Elevated non-esterified fatty acids and b-hydroxybutyrate and their association with transition dairy cow performance. Vet. J. 198:569-570.

Oltenacu, P.A., Broom, D.M., 2010. The impact of genetic selection for increased milk yield on the welfare of dairy cows. Anim. Welfare 19:39-49.

Penasa, M., Tiezzi, F., Sturaro, A., Cassandro, M., De Marchi, M., 2014. A comparison of the predicted coagulation characteristics and composition of milk from multi-breed herds of Holstein-Friesian, Brown Swiss and Simmental cows. Int. Dairy J. 35:8-10.

Piani, B., Fabro, C., Susmel, P., 2004. Measurement of purine derivatives and creatinine in urine by HPLC. In: H.P. Makkar and X.B. Chen (eds.) Estimation of microbial protein supply in ruminants using urinary purine derivatives. Kluwer Academic Publ., Dordrecht, The Netherlands, pp 149-166.

Pritchard, T., Coffey, M., Mrode, R., Wall, E., 2013. Genetic parameters for production, health, fertility and longevity traits in dairy cows. Animal 7:34-46.

Sgorlon, S., Colitti, M., Asquini, E., FERRARINI, A., Pallavicini, A., Stefanon, B., 2012. Administration of botanicals with the diet regulates gene expression in peripheral blood cells of Sarda sheep during ACTH challenge. Domest. Anim. Endocrin. 43:213-226.

Snedders, S., Dillon, P.G., O’Farrell, K.J., Diskin, M., Wylie, A.R., O’Callaghan, D., Rath, M., Boland, M.P., 2001. Genetic merit for milk production and reproductive success in dairy cows. Anim. Reprod. Sci. 65:17-31.

Sobhanirad, S., Carlson, D., Bahari Kashani, R., 2010. Effect of zinc methionine or zinc sulfate supplementation on milk production and composition of milk in lactating dairy cows. Biol. Trace Elem. Res. 136:48-54.

SPSS, 1997. Statistical package for social science, advanced statistics 7.5. SPSS Inc., Chicago, IL, USA.

Stefanon, B., Sgorlon, S., Gabai, G., 2005. Usefulness of nutraceuticals in controlling oxidative stress in dairy cows around parturition. Vet. Res. Commun. 29:387-390.

Stefanon, B., Volpe, V., Moscardini, S., Gruber, L., 2001. Using artificial neural networks to model the urinary excretion of total and purine derivative nitrogen fractions in cows. J. Nutr. 131:3307-3315.

Susmel, P., Stefanon, B., Spanghero, M., Mills, C.R., 1995. Daily variation of purine derivatives concentration in urine of cows fed once or twice daily. Zoot. Nutr. Anim. 21:145-153.

Viitala, S.M., Schulman, N.F., de Koning, D.J., Elo, K., Kinos, R., Virta, A., Virta, J., Makitaniila, A., Vilikki, J.H., 2003. Quantitative trait loci affecting milk production traits in Finnish Ayrshire dairy cattle. J. Dairy Sci. 86:1828-1836.