Defining a nitrogen efficiency index in Holstein cows and assessing its potential effect on the breeding program of bulls

Y. Chen,[1] H. Atashi,[1,2] C. Grelet,[3] S. Vanderick,[1] H. Hu,[1] and N. Gengler[1]*
[1]TERRA Teaching and Research Center, University of Liège, Gembloux Agro-Bio Tech (ULiège-GxABT), 5030 Gembloux, Belgium
[2]Department of Animal Science, Shiraz University, 71441-65186 Shiraz, Iran
[3]Walloon Agricultural Research Center (CRA-W), 5030 Gembloux, Belgium

ABSTRACT

The purposes of this study were (1) to explore the relationship between 3 milk mid-infrared predicted features including nitrogen intake (NINT), milk true protein N (MTPN), and milk urea-N yield (MUNY); (2) to integrate these 3 features into an N efficiency index (NEI) and analyses approximate genetic correlations between the NEI and 37 traits (indices) of interest; and (3) to assess the potential effect of including the NEI into breeding programs of bulls. The edited data were 1,043,171 test-day records on 342,847 cows in 1,931 herds and 143,595 test-day records on 53,660 cows in 766 herds used for estimating breeding values (EBV) and variance components, respectively. The used records were within 5 to 50 d in milk. The records were grouped into primiparous and multiparous. The genetic parameters for the included mid-infrared features and EBV of the animals included in the pedigree were estimated using a multiple-trait repeatability animal model. Then, the EBV of the NINT, MTPN, MUNY were integrated into the NEI using a selection index assuming weights based on the N partitioning. The approximate genetic correlations between the NEI and 37 traits of interest were estimated using the EBV of the selected bulls. The bulls born from 2011 to 2014 with NEI were selected and the NEI distribution of these bulls having EBV for the 8 selected traits (indices) was checked. The heritability and repeatability estimates for NINT, MTPN, and MUNY ranged from 0.09 to 0.13, and 0.37 to 0.65, respectively. The genetic and phenotypic correlations between NINT, MTPN, and MUNY ranged from −0.31 to 0.87, and −0.02 to 0.42, respectively. The NEI ranged from −13.13 to 12.55 kg/d. In total, 736 bulls with reliability ≥0.50 for all included traits (NEI and 37 traits) and at least 10 daughters distributed in at least 10 herds were selected to investigate genetic aspects of the NEI. The NEI had positive genetic correlations with production yield traits (0.08–0.46), and negative genetic correlations with the investigated functional traits and indices (−0.71 to −0.07), except for the production economic index and functional type economic index. The daughters of bulls with higher NEI had lower NINT and MUNY, and higher MTPN. Furthermore, 26% of the bulls (n = 50) with NEI born between 2011 to 2014 had higher NEI and global economic index than the average in the selected bulls. Finally, the developed NEI has the advantage of large-scale prediction and therefore has the potential for routine application in dairy cattle breeding in the future.

Key words: N intake, genetic correlation, health, mid-infrared spectrum

INTRODUCTION

The economic importance of genetic improvement for efficiency traits in cattle is recognized by the world's cattle producers (Brito et al., 2020). Measuring and improving efficiency is not only beneficial to the protection of the environment, but also beneficial to the farm, can promote the sustainable economic development of dairy production (Chen et al., 2021c), and strengthen its social acceptability. In the process of studying efficiency, researchers put forward various indicators representing dairy cow efficiency which can be defined very broadly. In the context of direct efficiency of a given animal, efficiency is often linked to feeding efficiency (**FE**) that can be divided further (i.e., into energy and nitrogen efficiency). Often FE is associated with the energy [e.g., energy intake (**EI**), energy balance (**EB**)] and an expression as residual trait compared with expectations [e.g., residual feed intake (**RFI**)] is used. Many works for FE and energy have been done (McParland et al., 2015; Brito et al., 2020), especially FE has started being used in the dairy cattle breeding system in some countries (e.g., Australia, the United States; Pryce et al., 2014; Parker Gaddis et al., 2021). However, there are relatively few studies on N use efficiency (**NUE**) of dairy cows, except for those addressing milk urea con-

centration (**MU**) or MUN as implicit proxies (Bobbo et al., 2020; Chen et al., 2021b). To our knowledge, only we have recently performed the genetic analyses of predicted NUE for dairy cows in early lactation (Chen et al., 2021c). Moreover, the NUE by cattle is very low (20–44%) and could be improved (Cantalapiedra-Hijar et al., 2018; Grelet et al., 2020). For example, recently Spanghero and Kowalski (2021) conducted a meta-analysis of 86 N balance experiments carried out in the past 20 yr, and found that the average NUE in cattle was 27% and more than half of the feed N is excreted through urine and feces.

As often for novel traits, the definition of NUE can be very different. Based on Calsamiglia et al. (2010), Grelet et al. (2020) defined NUE as milk N divided by the N intake (**NINT**). This definition has several shortcomings. First, from a mathematical point of view changes in ratios are highly unpredictable as they can come from changes in the denominator or the numerator. Moreover, as mentioned by Grelet et al. (2020), this definition for NUE does not account for the actual N losses through urine and feces, making it impossible to calculate the N balance. Consequently, early lactation cows having limited intake capacities and producing high quantities of milk may experience a negative N balance on top of the negative EB. Looking only at NINT and milk N, the NUE would be artificially high. There is then a risk of confusing artificially high NUE with negative N balance, and trying to improve NUE may increase the difficulty induced by severe mobilization of body reserves. Additionally, our previous study found that the genetic correlation between predicted NUE and MU was close to 0 (Chen et al., 2021a). Based

on the above reasons, we aimed to build a new N efficiency index (**NEI**) considering the NUE and N losses at the same time to avoid these shortcomings (Figure 1). The NEI and its composition both differ from the predicted NUE trait of our previous study. The MUN yield (**MUNY**) is linearly proportional to the urinary urea-N excretion when defined as a quantity excreted (Wattiaux, 2015). So the NEI index can be built based on NINT, milk true protein N (**MTPN**), and MUNY. Among these 3 features, MTPN and MUNY can be easily measured using traits that are currently recorded as explained later. However, the NINT is a feature that is difficult and expensive to measure in routine. The composition of milk is affected by the animal diet, and milk mid-infrared (**MIR**) spectra can reflect the changes in milk composition, which suggests that MIR can indirectly reflect the composition of the diet. (Klaffenböck et al., 2017). Grelet et al. (2020) developed a NINT prediction equation for dairy cows based on milk MIR spectra, which have been already applied to the Walloon region of Belgium data set (Chen et al., 2021a). The MIR spectra is an inexpensive method for predicting features, and it has been applied to predict various traits in dairy cows (Grelet et al., 2021). At the same time, if a new trait is added to the breeding program, it is necessary to know its potential effects on other traits included in the selection index and proxies of NUE (e.g., MU).

The purposes of this research were (1) to estimate the genetic parameters and EBV of NINT, MTPN, and MUNY; (2) to integrate these 3 features into an NEI based on the selection index and investigate the approximate genetic correlations between the NEI and 37
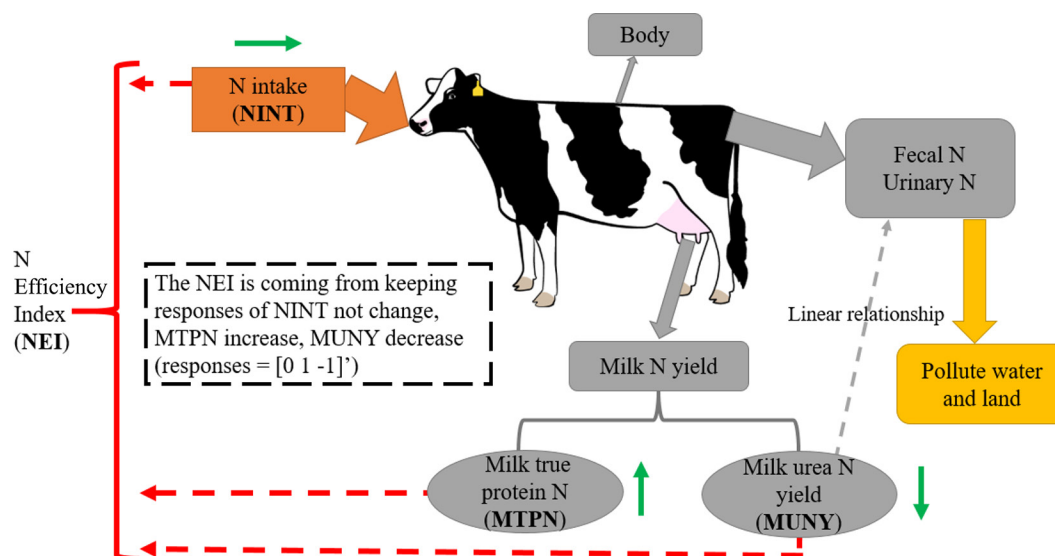

Figure 1. A new N efficiency index was built based on EBV from N intake, milk true protein N, and milk urea N yield.

traits of interest based on EBV of selected bulls; and (3) to assess the potential effect of including the NEI into breeding programs of bulls.

MATERIALS AND METHODS

Data

The data used in this research were collected from 2001 to 2019 by the official milk recording in the Walloon region of Belgium; thus, institutional animal care approval was not required. The milk samples were analyzed by MIR spectrometry (commercial instruments from FOSS) to assess milk compositions (including MU) and generate MIR spectra. Daily milk yield (**MY**), protein percentage (**PP**), and MU were restricted to the range of 1 to 90 kg, 1 to 7%, and 2 to 70 mg/dL, respectively. The filtering procedure used for NINT was the same as that explained by Chen et al. (2021c). In brief, 2 criteria are considered in the filtering procedure: (1) the standardized Mahalanobis distance between the new predicted spectrum and the calibration data set is ≤ 3, (2) the predicted value of NINT is within the range of 3 standard deviations (**SD**) of the mean. The formulas used to compute MTPN = [(MY $\times$ PP/6.38) − MUNY]; and MUNY = [(MU/2.14) $\times$ MY] (WHO and FAO, 2011) allowed to transform the concentration to yield. Furthermore, the range of DIM was restricted between 5 and 50, lactation number was restricted between one and 5, and calving age was restricted between 22 to 39, 34 to 53, 47 to 68, 59 to 83, and 71 to 98 mo for the first to fifth parity, respectively. At least 2 of the 3 included features had to be available. Finally, 1,043,171 test-day records on 342,847 cows in 1,931 herds collected between 2001 and 2019 remained. The pedigree related to the data set comprised 504,333 animals (17,573 males). In addition, each feature was divided into primiparous (n = 404,312) and multiparous (second to fifth parity; n = 638,859) classes. Hereafter, the features for NINT, MTPN, and MUNY are identified as **NINT1**, **MTPN1**, and **MUNY1** for primiparous cows, and **NINT2+**, **MTPN2+**, and **MUNY2+** for multiparous cows.

Milk MIR spectra of 53,660 cows in 776 herds were used leading to 143,595 NINT records. All milk MIR spectra were standardized according to Grelet et al. (2015). The NINT of each cow were predicted by the equation developed based on the models established by Grelet et al. (2020) using milk MIR spectra, MY, and parity as additional predictors, through support vector machine regression. The determination coefficient (R^2) and root mean square error of validation of the NINT model were 0.71 and 0.07 kg/d, respectively.

(Co)variance Components and Genetic Parameters

In total, 143,595 records (NINT1, MTPN1, MUNY1, NINT2+, MTPN2+, and MUNY2+) on 53,660 cows were used to estimate (co)variance components. The used pedigree included 132,056 animals (7,340 males). A 6-trait (3 features in 2 parity classes) repeatability animal model was used to estimate the (co)variance components. The used model was based on that presented by Chen et al. (2021c) to a different set of 6 traits:

$$\mathbf{y} = \mathbf{Hh} + \mathbf{Xb} + \mathbf{Qq} + \mathbf{W1c} + \mathbf{W2p} + \mathbf{Za} + \mathbf{e}, \quad [1]$$

where $\mathbf{y}$ was a vector of NINT1, MTPN1, MUNY1, NINT2+, MTPN2+, and MUNY2+. In each trait, all effects in this model were the same as Chen et al. (2021c). In brief, $\mathbf{h}$ was a vector of fixed effect of herd-year-season of calving; $\mathbf{b}$ was a vector of fixed regression coefficients for standardization DIM and its quadratic; $\mathbf{q}$ was a vector of fixed regression coefficients of the standardization age of calving, defined as a constant, linear and quadratic regression defined inside parities (from first to fifth parity); $\mathbf{c}$ was a vector of nongenetic cow random effect; $\mathbf{p}$ was a vector of nongenetic cow $\times$ parity random effect, this effect was only modeled for NINT2+, MTPN2+, and MUNY2+, as they allowed to distinguish records for the same cow but occurring during different parities (from second to fifth parity); $\mathbf{a}$ was a vector of the random additive genetic effect; $\mathbf{e}$ was a vector of random residual effect. In addition, $\mathbf{H}$, $\mathbf{X}$, $\mathbf{Q}$, $\mathbf{W1}$, $\mathbf{W2}$, and $\mathbf{Z}$ were incidence matrices assigning observations to effects.

The expected values and variances in Equation [1] were defined similarly to Chen et al. (2021c) but applied to a different set of 6 traits. The matrices of $V(\mathbf{c})$ and $V(\mathbf{a})$ both contained a block of 6×6 (co)variance matrices. For $V(\mathbf{e})$, the diagonal and off-diagonal elements of the matrix were nonzero and zero, respectively. For $V(\mathbf{p})$ the elementary (co)variance matrix was reduced to a 3×3 matrix, because only the (co)variances associated with NINT2+, MTPN2+, and MUNY2+ were present.

All computations were performed in the BLUPF90 programs (Misztal et al., 2018). The (co)variance components for NINT, MTPN, and MUNY were estimated by Gibbs sampling in THRGIBBS1F90 (version 2.118) through Equation [1], and posterior convergence was analyzed by POSTGIBBSF90 (version 3.14). Among them, the posterior means of (co)variances, heritabilities ($\mathbf{h^2}$), repeatability (**REP**), genetic and phenotypic correlations were obtained using 40,000 samples, which

is from a single chain of 220,000 after a burn-in of 20,000, and 1 in every 5 samples was saved. The formulas used to calculate the later 4 parameters were the same as those used by Chen et al. (2021c). Approximate standard errors (**SE**) of all calculated parameters were obtained using the POSTGIBBSF90 (version 3.14) program.

Estimated Breeding Value

A total of 1,043,171 records were used to estimate EBV for the included 6 traits based on the estimated (co)variance components. The EBV of the included traits were estimated according to a precondition conjugate algorithm implemented in the BLUP90IOD2 (version 3.81, http://nce.ads.uga.edu/wiki/doku.php?id=readme.pcg2) program using Equation [1]. Multi-trait reliability (**REL**) of EBV could not be calculated directly for the 6 included traits, therefore we divided the 6 included traits into 2-trait models (all effects were the same as Equation [1]) according to NINT, MTPN, and MUNY. This allows calculating the REL of EBV separately by direct inversion of the coefficient matrix for the NINT, MTPN, and MUNY. The 2-trait models were then solved using the BLUPF90 (version 1.70) program to extract diagonal elements of the inverted coefficient matrix allowing to compute REL in this way hereafter called **REL$_S$**. From this point, selection index theory was used based on a strategy proposed by VanRaden et al. (2018) to calculate REL. First, we restore the 6-trait REL as **REL$_M$**, the REL$_M$ assuming that the 6-trait solutions could have been obtained as index traits combing the 2-trait solutions. Needed regression coefficients were calculated using selection index theory:

$$\mathbf{B} = \mathbf{G}\mathbf{G}^{*-1},$$

where **G** was the full 6 × 6 matrix of the estimated genetic (co)variance of the explained 6-trait model, **G*** was equal to the **G** excluding the covariances between NINT, MTPN, and MUNY that were put to zero, and **B** was a 6 × 6 matrix of regression coefficients. Reliabilities of the 6 solutions were computed as the ratios between explained variances and total or maximum variances. Maximum (co)variances of 6-trait solutions were computed as follows:

$$\mathbf{V}_M = \mathbf{B}\mathbf{G}\mathbf{B}'.$$

In the next step, for each animal i, the matrix $\mathbf{R}_i$ was obtained by multiplying each element of **B** by the square root of the relevant element of REL$_S$ for this

animal. This allowed us to compute the (co)variances explained for this specific animal:

$$\mathbf{V}_{Si} = \mathbf{R}_i\mathbf{G}\mathbf{R}_i'.$$

Finally, with only the variance ratios being relevant, the following formula was used to obtain REL$_M$ for animal i:

$$\mathbf{REL}_{Mi} = \mathbf{diag}(\mathbf{V}_{Si})\mathbf{diag}(\mathbf{V}_M)^{-1}.$$

Nitrogen Efficiency Index

The NEI was calculated as follows:

$$\mathbf{NEI} = \mathbf{a}'\mathbf{u},$$

where **u** was a vector of EBV for the 6 included traits (NINT1, MTPN1, MUNY1, NINT2+, MTPN2+, and MUNY2+), and **a** was a vector of relative weights. Selection index theory for desired selection response was used to obtain the **a** coefficients by computing $\mathbf{a} = \mathbf{G}^{-1}\mathbf{r}_{org}$, where **G** was the 6 × 6 matrix of the genetic (co)variances for the 6 included traits, and $\mathbf{r}_{org}$ was the selection response vector of the 6 included traits. We assumed that the selection responses (**r**) for NINT, MTPN, and MUNY were 0, 1, –1 [$\mathbf{r} = (0\ 1\ -1)'$], respectively, which means keeping NINT unchanged, increasing MTPN, but decreasing MUNY. All traits were expressed in the same unit (kg/d); therefore, selection responses were defined in the original scale. As the **r** was defined for the 3 combined features across the 2 parity classes, a transformation matrix **T** was needed to convert **G** to **G$_t$**.

$$\mathbf{T} = \begin{bmatrix} 0.5 & 0 & 0 & 0.5 & 0 & 0 \\ 0 & 0.5 & 0 & 0 & 0.5 & 0 \\ 0 & 0 & 0.5 & 0 & 0 & 0.5 \end{bmatrix},$$

$$\mathbf{G}_t = \mathbf{T}\mathbf{G}\mathbf{T}',$$

$$\mathbf{a}_t = \mathbf{r}\mathbf{G}_t^{-1},$$

$$\mathbf{a} = \mathbf{a}_t\mathbf{T}.$$

The REL of NEI was calculated using the method given by VanRaden et al. (2018):

$$\mathbf{REL}_{NEI} = \frac{\mathbf{w}\mathbf{G}\mathbf{w}'}{\mathbf{a}\mathbf{G}\mathbf{a}'},$$

where $\mathbf{REL_{NEI}}$ was the REL of NEI and $\mathbf{w}$ was obtained by multiplying the elements of $\mathbf{a}$ by the square root of REL_M for the EBV of the 6 traits.

When we define $\mathbf{P}$ as the 6×6 matrix of the summed (co)variances for the 6 traits, h^2_{NEI} was the h^2 of NEI can be computed as

$$h^2_{NEI} = \frac{\mathbf{aGa'}}{\mathbf{aPa'}},$$

where $\mathbf{a}$ and $\mathbf{G}$ were explained previously.

Approximate Genetic Correlations

The approximate genetic correlations between the NEI and 37 traits of interest were estimated using the procedure proposed by Blanchard et al. (1983) based on the EBV of selected bulls. Those bulls that had NEI and had at least 10 daughters distributed in at least 10 herds, and their reliability of EBV for the NEI and 37 traits was at least 0.50, were selected. The SE of the estimated approximate genetic correlations was estimated using 1,000 bootstrapped replicates (Chen et al., 2021c). At the same time, the selected bulls were used to perform the same computation for the 6 traits included in the NEI; however, the reliability of the EBV of these 6 traits was required to be greater than 0.30 (Chen et al., 2021c).

In this study, we used EBV and its REL of 36 traits or indices (except for the MU) obtained by the national genetic evaluation system of dairy cows in the Walloon region of Belgium in April 2021 (https://www.elinfo.be/telechargerEN.html). The model and algorithm used for calculating EBV of the 36 traits of interest can be found in documents (Belgium) submitted to INTERBULL (https://interbull.org/ib/geforms) or reported by Vanderick et al. (2020). The EBV of MU and its REL were reported by Chen et al. (2021b), and we used the average daily EBV of the first 3 parities, which was expressed on a standardized scale with a mean of 100 and an SD of 10.

The 37 traits of interest were as follows: MU, MY, fat yield (**FY**), protein yield (**PY**), fat percentage (**FP**), PP, udder health (**UH**, which represents the opposite SCS), longevity (**LONG**), female fertility (**FF**), direct calving ease (**DCE**), maternal calving ease (**MCE**), production economic index, member economic index, capacity economic index, udder economic index, functional type economic index, functional economic index, global economic index, stature, chest width, body depth, rump angle, rump width, foot angle, rear leg set, rear leg rear view, udder depth, udder support, fore udder, front teat placement, teat length, rear udder height, rear teat placement, angularity, overall feet and leg score, overall udder score, and overall conformation score (**OCS**). The definitions of all indexes and their proportions to the global economic index have been explained by Vanderick et al. (2020).

Potential Effect of the Selection of NEI in Bulls

Two different approaches (phenotypic and genetic levels) were used to check the potential effect and possibility of selecting NEI in bulls.

In the first approach, we checked the phenotypes of cows. Based on EBV computed in the previous section, bulls with bottom 5% and top 5% NEI values were selected. Based on the newly selected bulls, their daughters were screened. The means and SD for the traits (MY, PP, MU, NINT, MTPN, MUNY) of these daughters were obtained by groups of bulls. The t-test was used to detect differences between the 2 groups for these 6 traits.

In the second approach, we showed the distribution between NEI and 8 traits or indices in bulls. Bulls born from 2011 to 2014 with reliability ≥0.50 for 8 selected traits or indices (MU, MY, PY, UH, and member, udder, functional, or global economic indices) from 37 traits (indices) and at least 10 daughters distributed in at least 10 herds were selected. The distribution between the NEI of the selected bulls and the new 8 selected traits (indices) was investigated. The paired trait distribution map was divided into 4 regions based on the average value of the traits (indices) of the selected bulls. The bulls that fall into the upper right corner are considered to be better bulls, except for MU (needed in the bottom right).

To make the NEI comparable to the selected traits (indices), NEI was standardized as follows:

$$NEI_{is} = \frac{NEI_i - \text{Mean}_{2015}}{SD_{2015}},$$

where NEI_{is} and NEI_i were, respectively, the standardized and nonstandardized NEI of i individual, Mean_{2015} and SD_{2015} were the average and SD of NEI from the cows born in 2015 (n = 17,597), respectively. Then, the NEI_{is} was expressed on a standardized scale with a mean of 100 and an SD of 10, which is the same as the LONG and other included functional traits (Vanderick et al., 2020). Additional data preparation and processing were done using R (https://r-project.org).

RESULTS AND DISCUSSION

Descriptive Statistics

The descriptive statistics of the traits involved in this study are presented in Table 1. The average, SD, and coefficient of variation (**CV**) of the included 6 traits in the primiparous were lower than those in the multiparous cows (parity 2+), except for the CV of NINT. The MUNY has the largest CV in primiparous and multiparous. The average of MTPN (0.13 kg/d) is about one-third of NINT (0.42 kg/d), which indicates that the NUE of the animals included in this research is about 33%. This value is within the range of 20 to 44% reported by Cantalapiedra-Hijar et al. (2018) and Grelet et al. (2020).

Genetic Parameter for 6 Traits

The variance components, h^2, and REP estimated for NINT1, MTPN1, MUNY1, NINT2+, MTPN2+, and MUNY2+ are presented in Table 2. The h^2 and REP of the 6 included traits ranged from 0.09 to 0.13 and 0.37 to 0.65, respectively. The genetic variances of the 6 included traits explained 12.30, 10.52, 12.95, 11.21, 11.48, and 9.22% of the corresponding total variances. For the 6 traits examined, as far as we know, this is the first report on their h^2 and rep. We compared the genetic parameters of these traits with those used for the

efficiency of different nutrition factors. Compared with MUNY, the MU(N) has higher h^2 and REP (Bobbo et al., 2020; Chen et al., 2021b). The NINT is similar to EI (energy efficiency) and DMI (FE). McParland et al. (2015) showed that h^2 and REP of EI predicted by MIR spectroscopy were 0.20 and 0.33, respectively. Li et al. (2016) reported that h^2 and REP of DMI in the first 4 weeks of lactation in Holstein cows were 0.26 and 0.68, respectively. The estimated h^2 of NINT was lower than that reported for EI and DMI, but the REP was somewhere in between.

Genetic and phenotypic correlations among the 6 included traits are presented in Table 3. As expected, the same feature has high genetic correlations between primiparous and multiparous (0.82–0.89). There were negative genetic correlations between NINT and MUNY (-0.31 to -0.16), and moderate positive genetic correlations were found between NINT and MTPN (0.40–0.51). The MTPN and MUNY showed moderate positive genetic correlations (0.37–0.54). However, the phenotypic correlations found between different features in the same parity class were relatively strong compared with those found for the same features between parity classes. The phenotypic correlations between NINT and MTPN in the primiparous and multiparous classes were, respectively, 0.34 and 0.42, indicating that genetic and phenotype correlations between NINT and MTPN are high. The phenotypic correlation estimated between MTPN and MUNY in the primiparous and

Table 1. Mean, SD, and CV of related features in genetic evaluation and (co)variance component estimation data sets

Parity class[1]	MY[2] (kg/d)	PP (%)	MU (mg/dL)	NINT (kg/d)	MTPN (kg/d)	MUNY (kg/d × 1,000)
Genetic evaluation data set (total n = 1,043,171)						
1 (n = 404,312)						
Mean	25.46	3.14	22.92	—[3]	0.12	2.73
SD	5.72	0.33	8.42	—	0.03	1.17
CV	22.49	10.40	36.75	—	22.68	43.05
2+ (n = 638,859)						
Mean	33.37	3.23	22.84	—	0.16	3.57
SD	8.11	0.39	8.85	—	0.04	1.63
CV	24.30	12.14	38.74	—	23.81	45.82
(Co)variance component estimation data set (total n = 143,595)						
1 (n = 44,321)						
Mean	26.60	3.15	23.40	0.42	0.13	2.92
SD	5.43	0.31	7.21	0.06	0.03	1.08
CV	20.41	9.75	30.81	14.84	20.31	36.99
2+ (n = 99,274)						
Mean	35.50	3.20	22.30	0.49	0.17	3.73
SD	7.80	0.36	7.87	0.07	0.04	1.57
CV	21.97	11.19	35.29	14.27	21.74	42.09

[1]Parity class: the parities (1–5) were divided into 2 classes based on primiparous (class 1) and multiparous (class 2+).

[2]MY = milk yield; PP = protein percentage; MU = milk urea concentration; NINT = N intake; MTPN = milk true protein N; MUNY = milk urea-N yield.

[3]NINT has 143,595 records.

Table 2. Heritability (h^2), repeatability, additive genetic variance $\left(\sigma_a^2\right)$, across-parity permanent environment (nongenetic cow) variance $\left(\sigma_c^2\right)$ (only for second and later lactations), within-parity permanent environment (nongenetic cow × parity) variance $\left(\sigma_p^2\right)$, and residual variance $\left(\sigma_e^2\right)$ of the proxies for traits in primiparous (n = 44,321) and multiparous (n = 99,274) Holstein cows

Trait[1]	h^2	Repeatability	σ_a^2	σ_c^2	σ_p^2	σ_e^2
NINT1 (100 g/d)	0.12 ± 0.01	0.37 ± 0.01	0.03 ± 0.00[2]	0.06 ± 0.00	NA[3]	0.14 ± 0.00
MTPN1 (100 g/d)	0.11 ± 0.01	0.60 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	NA	0.02 ± 0.00
MUNY1 (g/d)	0.13 ± 0.01	0.41 ± 0.01	0.10 ± 0.01	0.21 ± 0.01	NA	0.45 ± 0.01
NINT2+ (100 g/d)	0.11 ± 0.01	0.45 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.08 ± 0.00	0.18 ± 0.00
MTPN2+ (100 g/d)	0.11 ± 0.01	0.65 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
MUNY2+ (g/d)	0.09 ± 0.01	0.42 ± 0.00	0.15 ± 0.01	0.13 ± 0.01	0.41 ± 0.01	0.93 ± 0.01

[1]Trait: NINT1 = N intake in primiparous cows; MTPN1 = milk true protein N in primiparous cows; MUNY1 = milk urea N yield in primiparous cows; NINT2+ = N intake in multiparous cows; MTPN2+ = milk true protein N in multiparous cows; MUNY2+ = milk urea N yield in multiparous cows.

[2]SE is less than 0.005.

[3]NA = not applicable.

multiparous were 0.38 and 0.41, respectively. The phenotypic correlations estimated between the remaining trait pairs were relatively low (−0.02 to 0.20).

Nitrogen Efficiency Index

The h^2 of NEI is low (0.06), which is similar to that estimated for the predicted NUE (Chen et al., 2021c). It has been reported that h^2 for RFI, as an indicator of FE, in the first 60 DIM was 0.10 (Jamrozik et al., 2020) and that reported for from 50 to 250 DIM was 0.14 (Li et al., 2020). It has been shown that h^2 of RFI varied in different herds and countries (Tempelman et al., 2015). The NEI and its REL ranged from −13.13 to 12.55 kg/d, and 0.00 to 0.95, respectively. The mean REL of NEI of all animals included in the pedigree was 0.21 (SD = 0.11), which is similar to the REL of EBV of animals with RFI phenotype (Li et al., 2020). Li et al. (2020) evaluated the average REL of EBV for RFI for animals with genotypes and without phenotypes was only 0.13 (n = 1.6 million).

Although NEI has a low average REL, repeated predictions can be done cheaply. In this way, the REL of the NEI for bulls having more daughters can be increased. For example, the average REL of NEI for selected bulls in this study (n = 736) used for estimating the approximate genetic correlation was 0.68 (Figure 2).

Approximate Genetic Correlations Between the NEI and 37 Traits (Indices) of Interest

In total, 736 bulls with REL ≥0.50 for NEI and 37 traits (indices) of interest and at least 10 daughters distributed in at least 10 herds were selected for estimating the approximate genetic correlations between the NEI and the examined traits of interest. The distribution of the REL of the examined traits (indices) for the selected bulls is presented in Figure 2 and Supplemental Table S1 (https://github.com/Yansen0515/Defining_NEI_and_assessing_effect_on_bull). The average REL of all traits (indices) in the selected bulls were greater than 0.63, which guaranteed the reliability of our subsequent results. The countries of origin of the most bulls were the United States (235), Canada (130), and the Netherlands (123).

Table 3. Genetic correlations (above the diagonal) and phenotypic correlations (below the diagonal) among reported N-related traits in primiparous (n = 44,321) and multiparous (n = 99,274) Holstein cows

Trait[1]	NINT1	MTPN1	MUNY1	NINT2+	MTPN2+	MUNY2+
NINT1		0.48 ± 0.04	−0.31 ± 0.06	0.89 ± 0.03	0.40 ± 0.05	−0.25 ± 0.05
MTPN1	0.34 ± 0.00[2]		0.54 ± 0.05	0.51 ± 0.05	0.82 ± 0.03	0.46 ± 0.05
MUNY1	0.06 ± 0.01	0.38 ± 0.00		−0.16 ± 0.06	0.37 ± 0.05	0.87 ± 0.02
NINT2+	0.17 ± 0.01	0.13 ± 0.01	0.01 ± 0.01		0.49 ± 0.03	−0.19 ± 0.05
MTPN2+	0.11 ± 0.01	0.20 ± 0.01	0.11 ± 0.01	0.42 ± 0.00		0.50 ± 0.04
MUNY2+	−0.02 ± 0.01	0.09 ± 0.01	0.15 ± 0.01	0.14 ± 0.00	0.41 ± 0.00	

[1]Trait: NINT1 = N intake in primiparous cows; MTPN1 = milk true protein N in primiparous cows; MUNY1 = milk urea nitrogen yield in primiparous cows; NINT2+ = N intake in multiparous cows; MTPN2+ = milk true protein N in multiparous cows; MUNY2+ = milk urea nitrogen yield in multiparous cows.

[2]SE is less than 0.005.

The estimated approximate genetic correlations between NEI and the included traits (indices) are shown in Figure 3, and will be referred to as genetic correlations in the latter part of this article. As expected, the NEI and MU had a strong negative genetic correlation. This shows that increased NEI is associated with increased NUE and decreased N pollution. Positive genetic correlations were observed between NEI and yield traits (MY, FY, and PY), ranging from 0.08 to 0.46. The genetic correlation between NEI and FP was negative, but that found between NEI and PP was positive. This shows that an increased NEI is associated with increased production of N (MY and PY), a decreased FP, and an increased PP. This finding is consistent with the genetic relationships reported between predicted NUE and production by Chen et al. (2021c). This shows that in the current breeding system in the Walloon region of Belgium, increasing production traits will increase the NUE of cattle when other traits are not considered. The former studies also proved that cows with a higher FE had a higher milk production (Vallimont et al., 2011; Köck et al., 2018).

Unfavorable genetic correlations were observed between NEI and UH (-0.27). The predicted NUE and UH were reported to have a negative genetic correlation (Chen et al., 2021c). This means that efficiency traits and UH are negatively genetically correlated. Increased NEI led to increased milk production, subsequently increased the intensity of udder use, and reduced its health. The NEI had negative genetic correlations with LONG (-0.21) and FF (-0.24). The previous study found that cows with a higher FE had a longer day open (supports our results) and a longer production life (different from our results; Vallimont et al., 2013). Unfavorable genetic correlations were found between the NEI and calving ease traits (DCE, MCE). One explanation is that NEI and yield traits (MY, FY, and PY) were positively genetically correlated, but yield traits were negatively correlated with DCE.

The genetic correlation found between NEI and production economic index (index combined of MY, FY, PY, FP, and PP) was close to zero. Unfavorable genetic correlation was observed between the NEI and member economic index (-0.30, representing leg
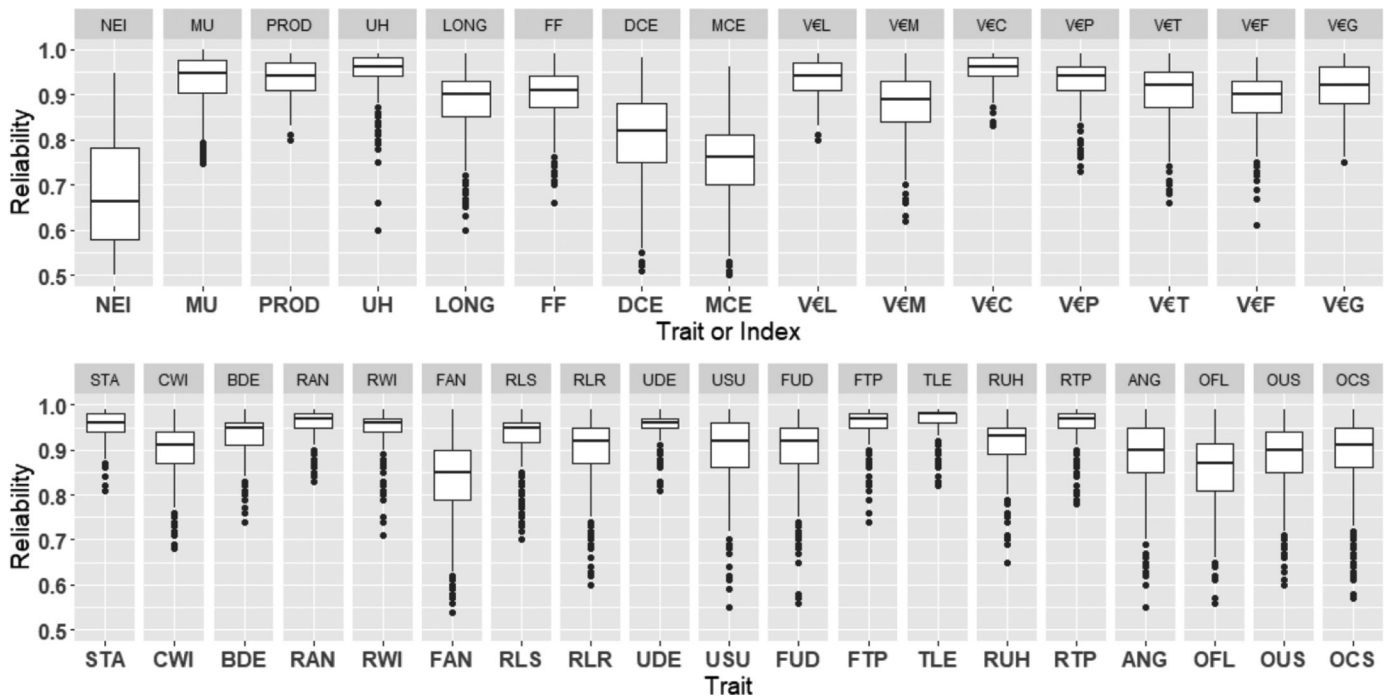

Figure 2. Distribution of reliability for bulls (n = 736) in nitrogen efficiency index (NEI), milk urea concentration (MU), production (PROD), udder health (UH), longevity (LONG), female fertility (FF), direct calving ease (DCE), maternal calving ease (MCE), production economic index (V€L), member economic index (V€M), capacity economic index (V€C), udder economic index (V€P), functional type economic index (V€T), functional economic index (V€F), global economic index (V€G), stature (STA), chest width (CWI), body depth (BDE), rump angle (RAN), rump width (RWI), foot angle (FAN), rear leg set (RLS), rear leg rear view (RLR), udder depth (UDE), udder support (USU), fore udder (FUD), front teat placement (FTP), teat length (TLE), rear udder height (RUH), rear teat placement (RTP), angularity (ANG), overall feet and leg score (OFL), overall udder score (OUS), and overall conformation score (OCS). Note: production includes milk yield, fat yield, protein yield, fat percentage, and protein percentage.
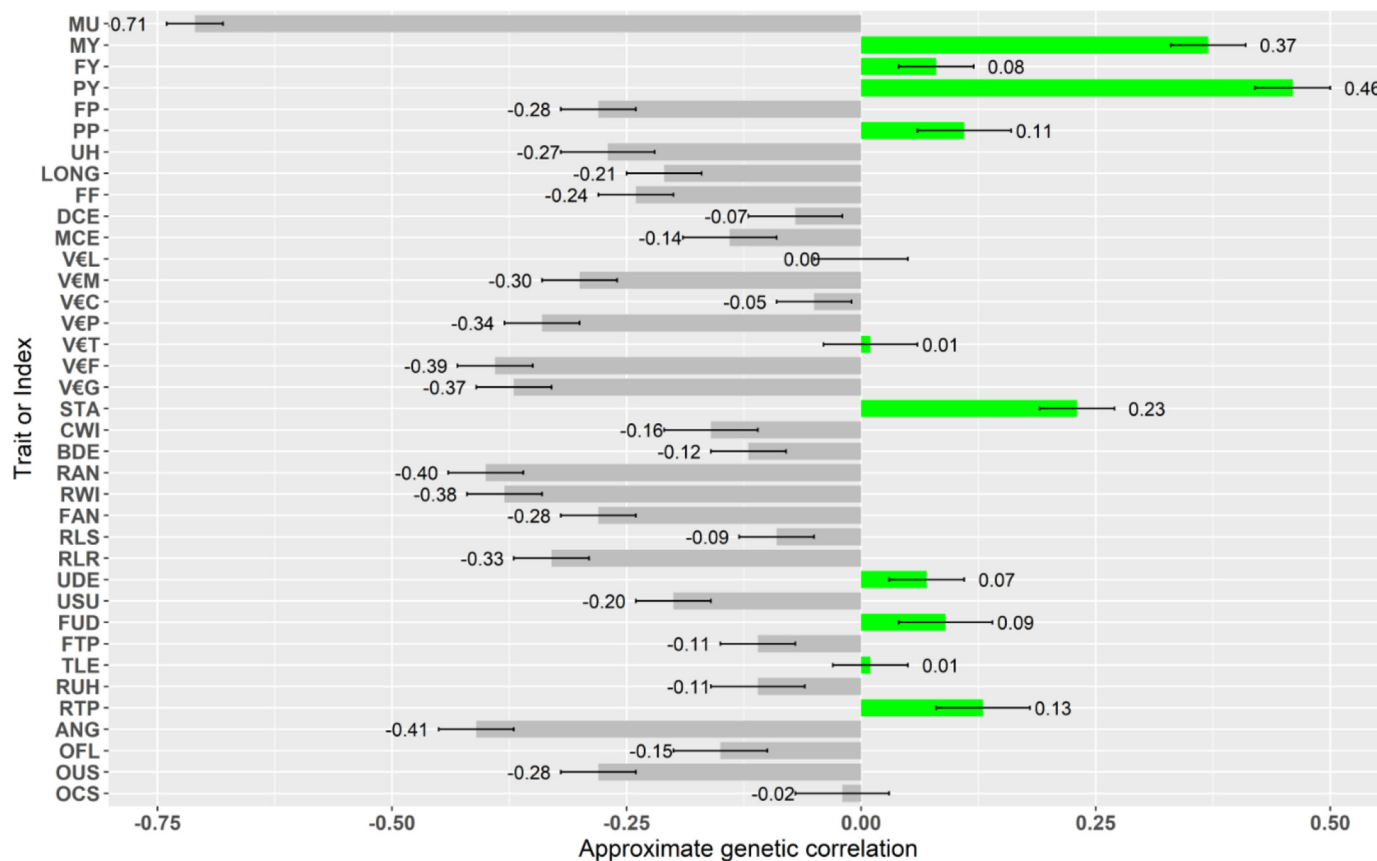
Figure 3. Approximate genetic correlation between nitrogen efficiency index (NEI) and other traits based on EBV of selected bulls (n = 736). Other traits included milk urea concentration (MU), milk yield (MY), fat yield (FY), protein yield (PY), fat percentage (FP), protein percentage (PP), udder health (UH), longevity (LONG), female fertility (FF), direct calving ease (DCE), maternal calving ease (MCE), production economic index (V€L), member economic index (V€M), capacity economic index (V€C), udder economic index (V€P), functional type economic index (V€T), functional economic index (V€F), global economic index (V€G), stature (STA), chest width (CWI), body depth (BDE), rump angle (RAN), rump width (RWI), foot angle (FAN), rear leg set (RLS), rear leg rear view (RLR), udder depth (UDE), udder support (USU), fore udder (FUD), front teat placement (FTP), teat length (TLE), rear udder height (RUH), rear teat placement (RTP), angularity (ANG), overall feet and leg score (OFL), overall udder score (OUS), and overall conformation score (OCS). All standard errors of approximate genetic correlation <0.05.

and hoof health). Köck et al. (2018) reported positive genetic correlations between FE (ECM/DMI), energy efficiency (energy in milk/EI), and the incidence of lameness, which is consistent with the findings of this study. Genetic correlation found between NEI and capacity economic index (representing body size) was low (−0.05). An unfavorable genetic correlation was observed between the NEI and udder economic index (−0.34, representing the udder health), the reason for this correlation is the same as we suggested for UH. The functional type economic index is a combination of member economic, capacity economic, and udder economic indices. But surprisingly, the genetic correlation estimated between NEI and functional type economic index was close to zero (0.01). The genetic correlation between NEI and functional economic index was negative, probably because functional economic index is a combination of UH, LONG, FF, DCE, and

MCE. The global economic index is a combination of 48% production economic index, 28% functional economic index, and 24% functional type economic index. Therefore, the NEI has a negative genetic correlation with global economic index. This means that the current in the Walloon region of Belgium used global economic index will not lead to a favorable correlated response of NUE.

The genetic correlations estimated between the NEI and the conformation traits ranged from −0.41 to 0.23, which also showed a mutually confirming relationship with the above results. Similar to the UH results, negative genetic correlations were found between NEI and rear udder height and overall udder score, suggesting that increased NEI is not conducive to improving udder health. The NEI and angularity were negatively genetically correlated. This result shows that NEI can cause cows to become fat, which is also consistent with

Table 4. Mean and SD of traits[1] for the daughters with bottom 5% and top 5% of nitrogen efficiency index sires

Group[1]	MY[2] (kg/d)	PP (%)	MU (mg/dL)	NINT (kg/d)	MTPN (kg/d)	MUNY (kg/d × 1,000)
Bottom 5% (n = 30,818)[3]						
Mean	31.30	3.18	24.20	0.48[4]	0.15	3.55
SD	8.21	0.36	8.60	0.08	0.04	1.59
Top 5% (n = 43,322)						
Mean	31.50	3.24	21.70	0.46	0.16	3.18
SD	8.45	0.37	8.12	0.08	0.04	1.48

[1]Group: P-value < 0.01 of the t-test between the 2 groups for all traits.

[2]MY = milk yield; PP = protein percentage; MU = milk urea concentration; NINT = N intake; MTPN = milk true protein N; MUNY = milk urea N yield.

[3]The 30,818 records in the bottom-5% group from 9,455 cows, and 43,322 records in the top-5% group from 13,506 cows.

[4]The 7,059 records for NINT in the bottom-5% group from 2,633 cows, and 13,389 records in the top-5% group from 4,683 cows.

the finding that NEI is not beneficial to the FF. The estimated positive genetic correlation between NEI and stature (0.23) combined with the above results suggests that cows with a higher NUE may have become taller and fatter. The NEI, rump angle, and rump width were negatively genetically correlated, which was conducive to the relationships between NEI, FF, and LONG of cattle. However, the genetic correlation found between NEI and OCS was close to zero (−0.02), indicating that the increasing NEI would not affect conformation traits.

The genetic correlations between 6 traits used in the NEI composition and 37 traits (indices) are shown in Supplemental Figures S1 to S3 (https://github.com/Yansen0515/Defining_NEI_and_assessing_effect_on__bull). The NINT had negative genetic correlations with MU, positive genetic correlations with production traits, functional traits, and global economic index, and its genetic correlations with OCS were close to zero. The genetic correlations of MTPN with MU were approximately 0, with production traits (except for FP), functional traits (except for UH and FF) and global economic index were positive, with OCS were negative. The MUNY had positive genetic correlations with MU, production traits (except for FP and PP), functional traits (except for FF), global economic index, and OCS.

In summary, NEI is genetically positively correlated with production traits but negatively correlated with the health, function, indices (except production economic and functional type economic indices), and most conformation traits, consistent with our previous study of the predicted NUE (Chen et al., 2021c).

Potential Effect of the Selected NEI in Bull

The bottom 5% and top 5% NEI bull groups each had 37 bulls. The mean and SD for 6 traits (MY, PP, MU, NINT, MTPN, MUNY) of their daughters were shown in Table 4. Means of the 6 included traits in daughters were significantly different in the 2 bull groups (all $P < 0.01$). Compared with the bottom-5% bull group, the NINT and MUNY were lower, and MTPN was higher in the top-5% bull group. The results showed that if we choose bulls with higher NEI, their daughters would have higher efficiency, which have more production and lower N pollution per N intake unit from these cows.

Distributions between NEI of the selected bulls (n = 50) born from 2011 to 2014 and EBV of the 8 selected traits (indices) are presented in Figure 4. Because the NEI has positive genetic correlations with MY and PY, we can easily find bulls with desirable NEI, MY, and PY at the same time (such as bull No. 45). The NEI has negative genetic correlations with the remaining 6 traits (indices), so a small number of bulls fell in the upper right corner of the distribution map (better bulls). The good news is that a small number of bulls can have better EBV for most of the traits, including NEI, at the same time (such as Bull No. 15). Overall, 26% of 50 bulls had both higher NEI and global economic index. This shows that using the NEI in genetic selection is feasible.

The NEI defined in this study may need further optimization. Although we considered NINT, MTPN, and MUN (instead of urine and feces N), the N consumption for maintenance of body metabolism was not included, and the MUNY is only an indicator of nitrogen losses, with limited accuracy. Therefore, in further steps we plan to add live weight or BCS to the existing NEI to improve it. The data of this study is based only on the first 50 DIM (limit from predicted NINT mode), which should be extended to the whole lactation. Currently, we only can use MU as an indicator for the whole lactation to indirectly increase the NUE and directly decrease the N pollution (Chen et
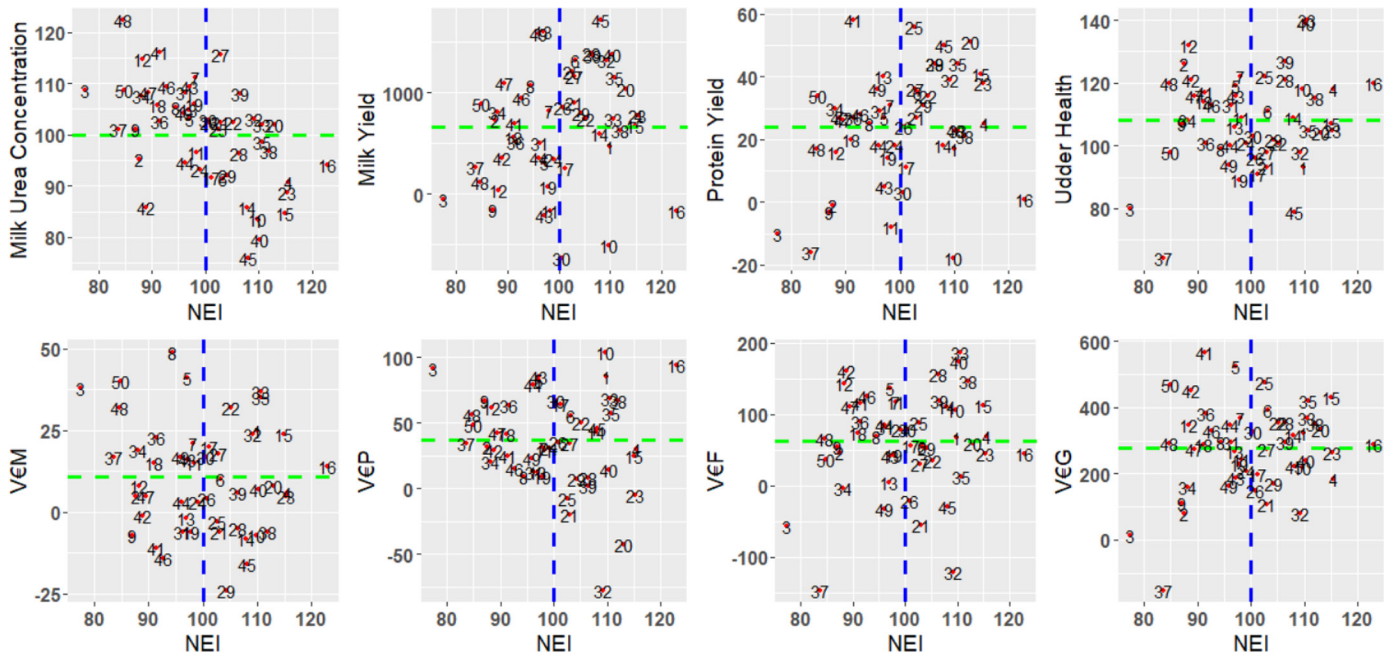
Figure 4. Distribution between nitrogen efficiency index (NEI) of bulls (n = 50) born from 2011 to 2014 and EBV of other 8 selected traits or indices (the dotted line represents the mean of the selection group). V€M = member economic index; V€P = udder economic index; V€F = functional economic index; V€G = global economic index.

al., 2021b). However, it should be noted that the genetic correlation between predicted NUE and MU was very low (Chen et al., 2021a). The NUE and MU are working in different processes of the NUE (Figure 1), which can explain, at least in part, why there is a low genetic correlation between predicted NUE and MU. The advantage of NEI is that it can consider both NUE and N pollution at the same time, and the genetic correlation between NEI and MU was −0.71. In addition, dairy cows in early lactation are in a state of negative EB. Because the utilization of N by dairy cows deeply depends on energy availability, the genetic relationship between NEI and the energy status of dairy cows still needs to be explored.

CONCLUSIONS

The findings of this study showed low h^2 for traits of NINT, MTPN, and MUNY, ranging from 0.09 to 0.13. The genetic correlations found among NINT, MTPN, and MUNY were positive, except for that found between NINT and MUNY. The NEI defined based on NINT, MTPN and MUNY has a low h^2 (0.06) and moderate REL (mean 0.21), but according to the range of NEI values (−13.13 to 12.55 kg/d), it has genetic selection potential. The NEI showed positive genetic correlations with production traits (e.g., MY, PY), ranging from 0.08 to 0.46. The genetic correlations estimated

between the NEI and MU, UH, LONG, FF, calving ease (DCE, MCE), and global economic index were negative, ranging from −071 to −0.07. Compared with the bottom 5% bull group, the NINT and MUNY were lower, and MTPN was higher in the top 5% bull group. The analyses of the selected bulls born from 2011 to 2014 showed that the bulls can have both higher NEI and global economic index than average at the same time. Finally, the developed NEI has the advantage of large-scale prediction and therefore has the potential to be routinely used in dairy cattle breeding in the future.

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ORCIDS

Y. Chen ● https://orcid.org/0000-0002-8593-4384
H. Atashi ● https://orcid.org/0000-0002-6853-6608
C. Grelet ● https://orcid.org/0000-0003-3313-485X
S. Vanderick ● https://orcid.org/0000-0003-4556-4718
H. Hu ● https://orcid.org/0000-0002-6408-1845
N. Gengler ● https://orcid.org/0000-0002-5981-5509