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Estimation of Nitrogen Use Efficiency for Ryegrass-Fed Dairy Cows: Model Development Using Diet- and Animal-Based Proxy Measures

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Abstract: This study aimed to identify suitable predictors of nitrogen (N) use efficiency (NUE; milk N/N intake) for cows that differed in breeds and were fed with ryegrass pasture, using existing data from the scientific literature. Data from 16 studies were used to develop models based on the relationships between NUE and dietary and animal-based factors. Data from a further 10 studies were used for model validation. Milk urea N (MUN) and dietary water-soluble carbohydrate-to-crudeprotein ratio (WSC/CP) were the best and most practical animal- and diet-based proxies to predict NUE. The results indicate that it might be necessary to adopt separate models for different breeds when using WSC/CP to predict NUE but not when using MUN.

Keywords: crude protein; Friesian; Jersey; milk urea nitrogen; soluble carbohydrates

1. Introduction

Prediction of nitrogen (N) use efficiency (NUE; milk N/N intake) of dairy cows at a herd or farm level is essential to support the development of sustainable dairy production, as it relates to the farm productivity, profitability, and environmental footprint [1–4]. In temperate grassland regions (e.g., Ireland), dairy cows are commonly grazed on pastures containing blends of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), which typically have high levels of N (ranges between 3.0 and 4.8% of DM) relative to dairy cow requirements for milk production [5]. This oversupply of dietary N results in low NUE and high (more than 50% of N intake) urinary N excretion, leading to increased N loss to the environment via nitrate leaching and nitrous oxide emission [5]. To estimate NUE of dairy cows, it is necessary to measure feed intake, which can be costly and difficult to accomplish with large numbers of grazing dairy cows. Therefore, there is an increased interest in estimating dairy cow NUE from easily obtained diet- and animal-based proxies [6,7].

Milk urea N (MUN) has been developed as a proxy measure of NUE in studies using mixed rations [8,9]. However, limited studies have investigated the usefulness of MUN to predict dairy cow NUE in pasture-based systems [10–12]. Previous studies indicated that NUE might be estimated from feed composition, such as the content of crude protein (CP) or water-soluble carbohydrates (WSC) [2,6]. However, these models were developed using a limited number of studies, and many earlier studies did not account for differences between breeds, despite that breed was shown to affect animal parameters such as MUN [13,14].

The study aimed to utilise data from the scientific literature and evaluate a range of diet- and animal-based measures since their value in predictive models for measuring NUE under pasture-based dairy cows. In addition, to examine the applicability of the resultant models to different cow breeds, we evaluated the models involving Friesian and Jersey × Friesian cows.

2. Material and Methods

2.1. Data Collection and Parameter Estimation

A literature search was conducted for the tenure (1996–2011) with the following keywords in Google Scholar: nitrogen use efficiency; ryegrass; cows; diet; milk urea; Friesian. Based on that search, studies between Friesian and Jersey × Friesian fed with a diet composed of more than 50% perennial ryegrass were selected in this study (Table 1). The studies were published between 1996 and 2011, and some unpublished data from 2013 were also made available by personal communication. Those studies were carried out at three locations of the South and North Island of New Zealand. The data obtained and used in this study were treatment means, with group sizes ranging between 3 and 30 cows (Table 1).

Table 1. Summary information about sources of data used in model development (Trial 1–16) and evaluation (Trial 17–26).

Study	Year	Groups of Cows in Study	Cows per Group	Breed	Region	Reference
1	1997	4	8	F	NZ	[15]
2	1999	3	15	F	NZ	[16]
3	2003	4	4	F	NZ	[17]
4	2009	14	20–30	F	NZ	[18]
5	2009	2	5	F	NZ	[19]
6	2010	8	15	F	NZ	[20]
7	1996	8	8	JF	NZ	[21]
8	1997	6	3	JF	NZ	[22]
9	1998	3	5	JF	NZ	[23]
10	2006	2	8	JF	NZ	[24]
11	2010	2	18	JF	NZ	[3]
12	2010	3	5	JF	NZ	Cheng unpublished 2010
13	2010	4	10	JF	NZ	[25]
14	2011	1	8	JF	NZ	[26]
15	2012	4	12	JF	NZ	[27]
16	2013	4	8	JF	NZ	Cheng unpublished 2013
17	2005	2	4–8	F	Netherlands	[28]
18	2006	3	4	F	Netherlands	[29]
19	2009	2	10	F	Netherlands	[30]
20	2013	4	8	F	Ireland	[31]
21	2010	3	16	F	NZ	[32]
22	2013	3	12	JF	NZ	[33]
23	2014	6	6	JF	NZ	[34]
24	2015	2	5	JF	NZ	[35]
25	2015	3	3	JF	NZ	[36]
26	2016	9	4	JF	NZ	[37]

(F—Friesian; JF—Jersey × Friesian).

The parameters extracted or calculated from the literature are summarised in Table 2, along with their units, abbreviations, and formulae used in calculations. Table 2 also shows the interrelationship of some of the variables and the derived measures often used to examine dairy system performance. Not all parameters listed in Table 2 were available or calculated across all studies since the required information was not provided.

Table 2. Variables measured, units, abbreviations, and formulae for their calculation. If formulae have not been provided, variables were measured directly by chemical analysis.

	Parameter	Abbreviation	Formula	Unit
Dietary factors	Metabolisable energy	ME		(MJ/kgDM)
	Neutral detergent fibre	NDF		(%DM)
	Acid detergent fibre	ADF		(%DM)
	Crude protein	CP		(%DM)
	Water-soluble carbohydrate	WSC		(%DM)
	Nitrogen	N		(%DM)
	Water-soluble carbohydrate to crude protein ratio		WSC/CP	(g/g)
	Metabolisable energy to crude protein ratio		ME/CP	(MJ/g)
Animal factors	Dry matter intake	DMI		(kg/cow/d)
	ME intake	MEI	ME x DMI	(MJ/cow/d)
	Milk yield	MY		(kg/cow/d)
	Milk nitrogen%	MN%		(% volume)
	Milk nitrogen	MN	MN% × MY × 10	(g/cow/d)
	Milk protein%	MP%		(% volume)
	Milk protein	MP	MN × 6.38/1000	(kg/cow/d)
	Milk fat%	MF%		(% volume)
	Milk fat	MF	MF% × MY/100	(kg/cow/d)
	Milk solids	MS	MP + MF	(kg/cow/d)
	Nitrogen intake	NI	DMI × N	(g/cow/d)
	Urinary nitrogen	UN		(g/cow/d)
	Faecal nitrogen	FN		(g/cow/d)
	Milk urea nitrogen	MUN		(mmol/L)
Derived measures	Nitrogen use efficiency	NUE	MN/NI	(g/g)
	Milk nitrogen to urinary Nitrogen ratio		MN/UN	(g/g)
	Urinary nitrogen to Nitrogen intake ratio		UN/NI	(g/g)
	Faecal nitrogen to urinary Nitrogen ratio		FN/UN	(g/g)
	Milk solids to dry matter Intake ratio		MS/DMI	(kg/kg)
	Milk yield to dry matter Intake ratio		MY/DMI	(kg/kg)
	ME intake to milk yield ratio		MEI/MY	(MJ/kg)
	ME intake to milk solid ratio		MEI/MS	(MJ/kg)

2.2. Statistical Analysis and Model Development

Statistical analyses were carried out using Genstat Version 15 (VSN International Ltd., Hemel Hempstead, UK). Summary statistics (means, standard error, minimum value, and maximum value) were calculated for each parameter using available group means. A stepwise process of model development was conducted, initially examining the basic correlations and later developing multiple regression and nonlinear mathematical functions. The sequence of model development is summarised as follows:

i. Rank correlations to examine the strength of monotonic relationships of each parameter with NUE, without making assumptions of the linearity of any relationships;
ii. Simple linear regression to identify linear relationships of the predictor parameters with NUE;
iii. For those parameters identified as having strong relationships with NUE from (i) and (ii), all subset regression procedures were performed to produce multiple regression models for NUE. These models were evaluated using Akaike information criterion (AIC) values and adjusted R^2 values, with high R^2 values and low AIC values indicat-

ing a high predictive ability [38]. The goodness of fit was also examined visually by plotting predicted values to the observed NUE obtained from each study;

iv. Parameters identified as being included in the best multiple regression models in (iii) were then screened for their convenience in terms of ease of measurement and practical use as proxy measures;

v. For the parameters identified from (iv), linear and nonlinear (asymptotic curves) models were fitted for both breeds combined and separately for each breed to assess whether models were breed specific or applicable across Friesians and Jersey × Friesian cows.

2.3. Rank Correlations and Simple Linear Regression Models

Since the initial screening process was conducted to examine the closeness of monotonic relationships between NUE and all the other parameters, rank correlation coefficients were calculated separately for Friesians and Jersey × Friesians. For any parameters showing significant rank correlations with NUE for both breeds and consistent relationships, simple linear regressions were performed. Both unweighted and weighted data were used to minimise the differences in the number of cows per group across studies (Table 1). There was no inclusion of 'study' as a random factor in these models since many of the covariates differed significantly between studies [39,40].

2.4. All Subset Regression Procedures and Multiple Regression Models

From the simple linear regression models, a further selection of parameters was performed based on statistical significance ($p < 0.01$) for both breeds in weighted and unweighted models and the number of cases available for both breeds greater than 10. From this process, six parameters were selected for further consideration, namely, CP, N, WSC/CP, N intake (**NI**), MUN, and MN/UN ratio. However, NI and MN are used in the calculation of NUE; thus, NI and MN/UN were judged as invalid predictors of NUE and not considered further.

Models were further evaluated for ease of measurement and practicality of application on the farm. From the all subset regression analysis, it was found that the animal-based parameter MUN and the dietary parameter WSC/CP produced the best estimates of NUE, which are independent variables and relatively easy for farmers to obtain.

2.5. Evaluation of WSC/CP and MUN as Predictors of NUE

A more detailed analysis of the relationship between NUE and WSC/CP and between NUE and MUN was performed. The relationships between NUE and WSC/CP were positive and linear, and therefore, simple linear regression models were fitted, comparing overall trends and the trends within each breed.

For MUN, the relationships with NUE were negative and asymptotic, and therefore, curves of the form $\text{NUE} = a + bc^{\text{MUN}}$, where a, b and c are constants, $c < 1$ and $(a + b)$ represents the maximum achievable NUE when MUN = 0. As these models included nonlinear functions, it was thought inappropriate to compare them using R^2 values [41]. Instead, the goodness of fit of the raw data to the models was assessed using S (the standard error of the observations or standard error of the regression) calculated as the square root of the mean square error term from the regression model.

2.6. Model Evaluation

To evaluate the models predicting NUE from MUN and WSC/CP, data were obtained from a further ten studies, five involving pure Friesian cows and five involving Jersey × Friesian cows (Table 1). Data involving Jersey × Friesian cows were obtained from recent studies (2013–2016) conducted in New Zealand. These were the only available studies that we could find in New Zealand in which cows were fed a diet composed of more than 50% perennial ryegrass. However, it was not possible to find recent results involving pure Friesian cows in New Zealand, and therefore, data were obtained from European countries with similar ryegrass-based dairy systems (The Netherlands and Ireland) (Table 1).

The observed values of NUE were compared with those predicted using the breed-specific linear models relating NUE with WSC/CP and with the combined breed exponential model relating NUE with MUN (Models 7, 8, and 10). Lin's concordance was used as a measure of correspondence between observed and predicted NUE values [42], and deviation of the observed NUE values (O_i) from those predicted (P_i) by the models was quantified in the following four ways:

(i) Mean absolute error, $MAE = \left(\sum_{i=1}^{n}|O_i - P_i|\right)/n$;

(ii) Mean relative absolute error, $MRAE = \left[\sum_{i=1}^{n}\left(\frac{|O_i - P_i|}{O_i}\right) \times 100^{\%}\right]/n$;

(iii) Root mean square error, $RMSE = \sqrt{\sum_{i=1}^{n}(O_i - P_i)^2/n}$;

(iv) Normalised root mean square error, $NRMSE$ = ($RMSE$/mean (Obs)) × 100%.

3. Results

3.1. The Database, Rank Correlations, and Simple Linear Regression Models

The data set covered a wide range of pasture quality (Table 3). For example, pasture CP content ranged from 11.6 to 28.0% of DM, metabolisable energy (ME) from 9.2 to 12.6 MJ/kg DM, neutral detergent fibre (NDF) from 34 to 59% of DM, and WSC from 5 to 34% of DM. The range of animal DMI was slightly larger for Jersey × Friesians (10–19 kg/cow/d) than for Friesians (12–17 kg DM/cow/d); however, the mean DMI was similar between the breeds (14.4 vs. 14.2 kg DM/cow/d for Friesians and Jersey × Friesians, respectively).

In the simple linear regressions, similar constants and slopes were obtained when using weighted or unweighted models (Table 4), and the directions of relationships with NUE were consistent between the two breeds. Of all single dietary factors, WSC/CP was the most accurate predictor of NUE, producing high adjusted R^2 values for both breeds (weighted models produced adjusted R^2 = 77.0% and 72.4% for Friesians and Jersey × Friesians, respectively).

3.2. All Subset Regression Analyses and Multiple Regression Models

A subset regression procedure was performed with MUN, WSC/CP, N, and BREED as explanatory factors, with models being ranked in order of predictive ability using both adjusted R^2 and AIC values (Table 5). The model with the highest R^2 (and lowest AIC) included only MUN and WSC/CP (Models 1 and 2; Table 5). Forcing BREED into these models resulted in only a minor change in R^2 and AIC values (Models 3 and 4). The best model (based on AIC) when BREED was forced into the regression model omitted WSC/CP and contained only MUN and BREED (Model 5). However, when all available data for this model were used, the adjusted R^2 dropped to only 56.8% (Model 6; Table 5).

3.3. Practical Parameters: The Relationships of NUE with WSC/CP and MUN

The relationship between NUE and WSC/CP was linear (Table 6; Figure 1). However, a model based on the data from both breeds of cows was not a good fit and produced a relatively high standard error of the observations (Table 6; Model 7). The interaction term between WSC/CP and BREED in a general linear model was highly significant ($F_{1,41}$ = 19.27; $p < 0.001$), suggesting that separate models for NUE vs. WSC/CP should be fitted to the different breeds (Figure 1). In support of this, the 95% CIs of the coefficients in the separate breed linear models relating NUE vs. WSC/CP showed no overlap (Models 8 and 9; Table 6).

Table 3. Summary of parameters for ryegrass-fed Friesian and Jersey × Friesian dairy cows used in this analysis. N = the number treatment means used in the calculation of the summary statistics for each parameter.

		Friesian							**Jersey × Friesian**						
		N	**Mean**	**SE**	**Min**	**Max**	r_S	**Sig.**	**N**	**Mean**	**SE**	**Min**	**Max**	r_S	**Sig.**
Dietary factors	ME (MJ/kgDM)	9	11.51	0.41	9.16	12.59	0.70	***	37	11.80	0.09	9.87	12.70	0.45	***
	NDF (%)	28	45.67	1.47	33.80	59.00	0.04	ns	31	43.22	0.93	34.20	58.90	−0.28	*
	ADF (%)	8	26.79	0.95	23.88	32.30	0.25	ns	18	24.12	1.02	18.70	32.20	−0.02	ns
	CP (%)	29	20.00	0.89	11.88	28.00	−0.52	***	37	18.62	0.64	11.60	25.30	−0.61	***
	WSC (%)	22	18.62	1.15	13.90	34.20	0.70	***	24	19.23	0.99	5.00	28.70	0.13	ns
	WSC/CP (g/g)	22	0.948	0.114	0.496	2.590	0.71	***	23	1.092	0.068	0.674	1.780	0.57	***
	ME/CP(MJ/g)	9	0.731	0.051	0.537	0.928	0.90	***	37	0.664	0.026	0.489	1.034	0.75	***
Animal factors	MEI (MJ/cow/d)	9	170.39	8.53	120.00	196.44	0.45	ns	37	167.81	5.95	116.20	229.20	0.03	ns
	DMI (kg/cow/d)	29	14.40	0.27	12.28	16.60	0.43	**	37	14.20	0.47	10.20	19.10	−0.07	ns
	MY (kg/cow/d)	21	19.25	1.06	10.60	28.70	0.51	**	27	17.40	0.81	9.92	24.90	−0.06	ns
	MN (%)	21	0.544	0.012	0.461	0.620	−0.66	***	27	0.600	0.012	0.511	0.706	0.70	***
	MN (g/cow/d)	35	105.42	3.52	58.32	160.00	0.24	*	37	99.14	3.91	55.82	150.00	0.38	**
	MP (kg/cow/d)	35	0.677	0.022	0.372	1.021	0.24	*	31	0.644	0.025	0.356	0.957	0.31	*
	MF (%)	13	4.141	0.343	1.530	5.490	−0.28	ns	27	5.367	0.113	4.170	6.240	0.31	*
	MF (kg/cow/d)	13	0.706	0.052	0.313	0.930	0.36	ns	27	0.889	0.028	0.545	1.210	0.05	ns
	MS (kg/cow/d)	13	1.293	0.067	0.915	1.586	0.29	ns	27	1.542	0.053	0.901	1.944	0.22	ns
	NI (g/cow/d)	35	464.4	17.1	283.2	650.0	−0.40	**	37	420.0	19.5	201.6	616.0	−0.50	***
	UN (g/cow/d)	18	240.9	20.1	81.0	343.0	−0.81	***	14	247.8	15.4	165.0	357.9	0.24	ns
	FN (g/cow/d)	4	121.75	4.19	114.00	129.00	0.65	ns	14	110.86	4.36	86.00	138.00	0.37	*
	MUN (mmol/L)	35	12.22	0.77	4.00	17.90	−0.71	***	33	10.10	0.78	3.17	17.60	−0.81	***
Derived measures	MN/UN (g/g)	18	0.515	0.058	0.279	1.210	0.93	***	14	0.379	0.021	0.250	0.527	0.82	***
	UN/NI (g/g)	18	0.510	0.025	0.274	0.663	−0.84	***	14	0.538	0.021	0.458	0.722	−0.42	*
	MS/DMI (kg/kg)	13	0.085	0.004	0.058	0.108	0.27	ns	27	0.108	0.003	0.069	0.138	0.52	***
	MY/DMI (kg/kg)	21	1.273	0.057	0.809	1.750	0.48	**	27	1.197	0.030	0.834	1.515	0.35	*
	MEI/MY (MJ/kg)	9	10.52	0.35	9.13	12.06	−0.50	**	27	10.02	0.21	7.86	12.16	−0.31	*
	MEI/MS (MJ/kg)	9	126.48	2.52	115.85	138.56	−0.18	ns	27	112.23	2.98	89.41	143.09	−0.53	***
	NUE (g/g)	35	0.231	0.006	0.178	0.331			37	0.249	0.013	0.158	0.468		

r_s—Spearman's rank correlation for the relationship between each parameter and NUE. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns—not significant.

Table 4. Linear regression equations (coefficients (SE)) for the relationships between NUE and selected parameters for ryegrass-fed Friesian and Jersey × Friesian dairy cows. N = number of group means used in each analysis. Models are given for both unweighted and weighted group means.

			Friesian					Jersey × Friesian				
		Weighted	**N**	**Constant**	**Slope**	**Adj. R^2 (%)**	***p***	**N**	**Constant**	**Slope**	**Adj. R^2 (%)**	***p***
Dietary factors	CP (%)	No	29	0.326 (0.025)	−0.0047 (0.0012)	32.3	<0.001	37	0.533 (0.042)	−0.0153 (0.0022)	55.9	<0.001
		Yes	29	0.323 (0.025)	−0.0044 (0.0012)	33.0	<0.001	37	0.597 (0.041)	−0.0186 (0.0023)	64.8	<0.001
	WSC/CP (g/g)	No	22	0.170 (0.009)	0.0665 (0.0084)	74.4	<0.001	23	0.020 (0.043)	0.2144 (0.0377)	58.8	<0.001
		Yes	22	0.166 (0.009)	0.0735 (0.0087)	77.0	<0.001	23	0.020 (0.036)	0.2318 (0.0303)	72.4	<0.001
Animal factors	NI (g/cow/d)	No	35	0.317 (0.027)	−0.0002 (0.0001)	22.1	0.003	37	0.417 (0.039)	−0.0004 (0.0001)	34.3	<0.001
		Yes	35	0.328 (0.028)	−0.0002 (0.0001)	27.1	<0.001	37	0.480 (0.036)	−0.0005 (0.0001)	50.4	<0.001
	MUN (mmol/L)	No	35	0.312 (0.011)	−0.0067 (0.0009)	63.2	0.001	33	0.403 (0.021)	−0.0147 (0.0019)	63.7	<0.001
		Yes	35	0.324 (0.009)	−0.0075 (0.0007)	77.6	<0.001	33	0.426 (0.021)	−0.0175 (0.0022)	66.1	<0.001
Derived measures	MN/UN (g/g)	No	18	0.150 (0.010)	0.1725 (0.0182)	83.9	<0.001	14	0.100 (0.017)	0.2593 (0.0436)	72.6	<0.001
		Yes	18	0.138 (0.012)	0.2023 (0.0234)	81.3	<0.001	14	0.096 (0.019)	0.2659 (0.0480)	69.5	<0.001

Table 5. Multiple regression models (coefficients (SE)) for NUE produced by all subset regression procedures. The BREED factor was 'forced' into Models 3−6. N = number of groups means used in each analysis. Some models are given for both unweighted and weighted data. Models 5 and 6 involve the same parameters but using either (Model 5) the same 45 samples used in models 1–4 or (Model 6) all the data available for these parameters.

Model	Breed	Weighted	Constant	MUN (mmol/L)	WSC/CP	Breed (JF)	N	Adj. R^2 (%)	AIC	p
1	No	No	0.3326 (0.0408)	−0.0102 (0.0019)	0.0297 (0.0204)	-	45	66.6	47.03	<0.001
2	No	Yes	0.3238 (0.0436)	−0.0096 (0.0020)	0.0381 (0.0225)	-	45	71.1	47.28	<0.001
3	Yes	No	0.3402 (0.0428)	−0.0104 (0.0020)	0.0292 (0.0205)	−0.0082 (0.0126)	45	66.1	48.00	<0.001
4	Yes	Yes	0.3215 (0.0475)	−0.0095 (0.0022)	0.0385 (0.0230)	0.0018 (0.0137)	45	70.4	48.26	<0.001
5	Yes	No	0.3943 (0.0199)	−0.0124 (0.0014)	-	−0.0088 (0.0128)	45	65.3	47.98	<0.001
6	Yes	No	0.3591 (0.0157)	−0.0105 (0.0011)	-	0.0018 (0.0104)	67	56.8	-	<0.001

Table 6. Models (coefficients (95% CI)) relating NUE of ryegrass-fed Friesian and Jersey × Friesian cows to the dietary parameter WSC/CP and animal-based parameter MUN. Models are given for all the available data and each breed separately. S is the standard error of the observations.

	Equation of Form: NUE = $a + b$(WSC/CP)						
Model	**Breed**	a	b		**N**	**S**	p
7	All	0.1324 (0.093–0.172)	0.1087 (0.073–0.144)		45	0.052	<0.001
8	F	0.1700 (0.151–0.189)	0.0665 (0.049–0.084)		22	0.021	<0.001
9	J-F	0.0195 (−0.070–0.109)	0.2144 (0.136–0.293)		23	0.058	<0.001
	Equation of Form: NUE = $a + bc^{\mathrm{MUN}}$						
		a	b	c	**N**	**S**	p
10	All	0.2001 (0.187–0.211)	0.7000 (0.502–1.003)	0.7085 (0.645–0.769)	68	0.028	<0.001
11	F	0.1906 (−0.087−0.212)	0.2506 (−0.051–0.559)	0.8393 (0.704–0.979)	35	0.022	<0.001
12	J-F	0.1896 (0.163–0.210)	0.6950 (0.486–1.053)	0.7306 (0.647–0.807)	33	0.031	<0.001

Examination of all models weighted and unweighted, with and without BREED, shows little difference in coefficients between models. In addition, plots of predicted NUE values against observed NUE values (Figure 2) revealed a relatively equal distribution of residuals from the line of best fit, except for one or two groups of Friesian cows that had lower than predicted NUE values and a cluster of Jersey × Friesian groups that had higher NUE values than predicted by the models.

The negative relationship between NUE and MUN was best described by an asymptotic curve (Table 6; Figure 3). Unlike WSC/CP, an all-inclusive model (Model 10) produced a relatively low value of S of 0.028 (Table 6), and the 95% CIs for all the model parameters overlapped for the individual breed models and the model based on both breeds. The separate models for the Friesian and the Jersey × Friesian cows appeared to differ in only one aspect—coefficient b when the equation was written as NUE = $a + bc^{\mathrm{MUN}}$. This coefficient partially dictates the intercept of the line on the y-axis ($a + b$, i.e., the NUE when MUN = 0) and was 0.25 g/g for Friesian and 0.69 g/g for Jersey × Friesian cows.

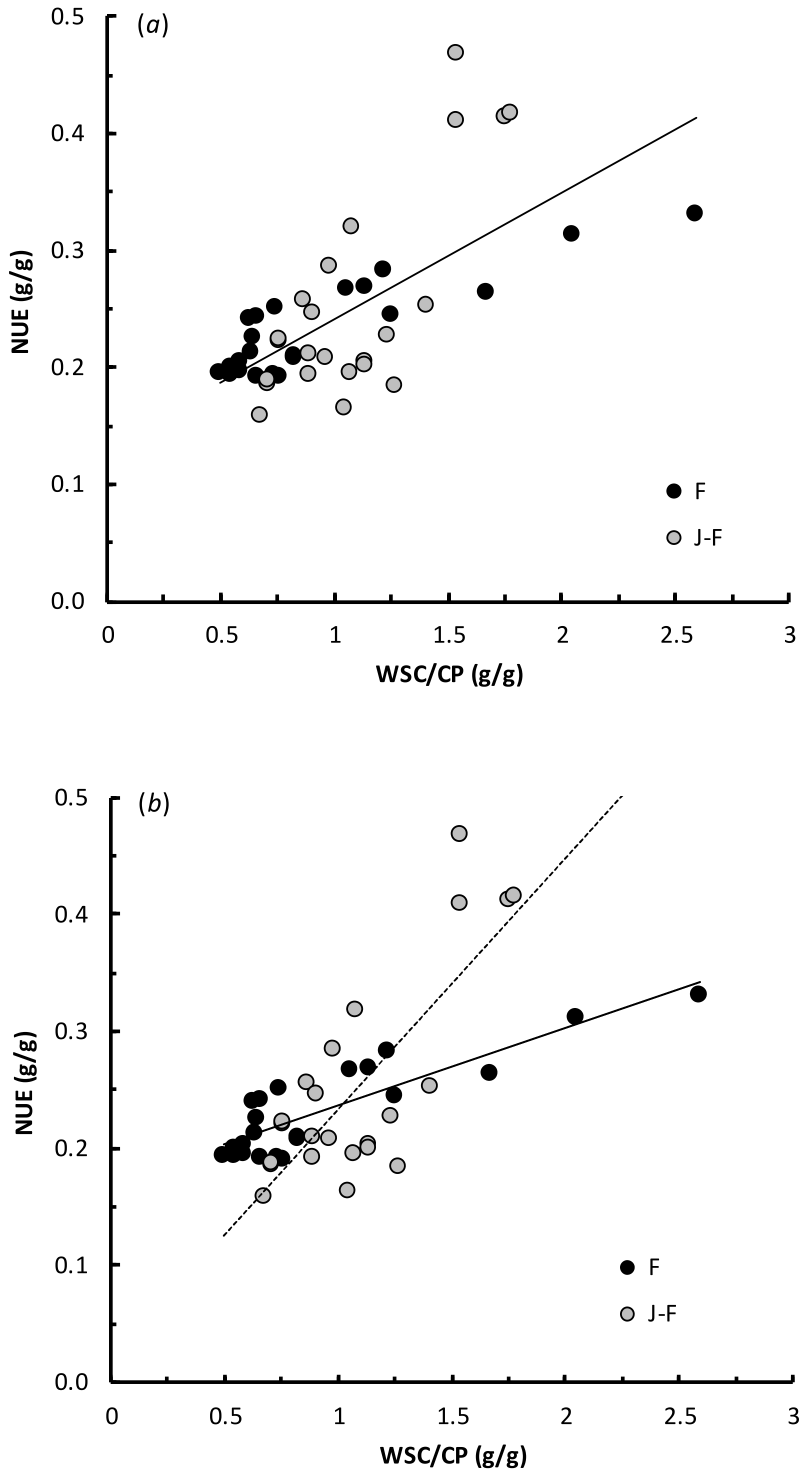

Figure 1. Linear relationships between NUE and dietary WSC/CP ratio for groups of ryegrass-fed Friesian (N = 22) and Jersey × Friesian (N = 23) cows: (**a**) shows the overall line of best fit (Model 7; Table 6) and (**b**) shows separate regression models for both breeds (Models 8 and 9; Table 6).

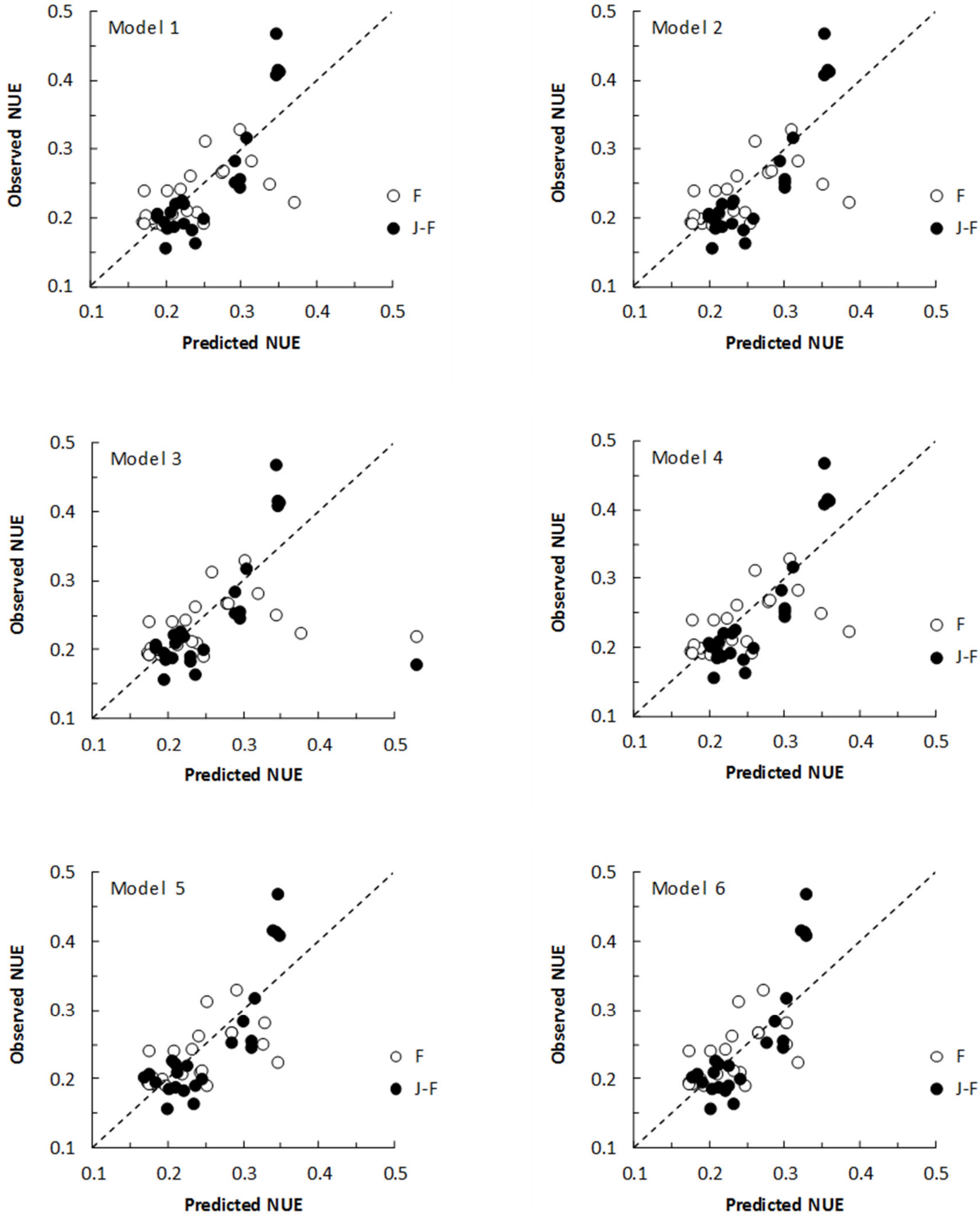

Figure 2. Plots of predicted NUE values vs. observed group NUE scores for Friesian and Jersey × Friesian cows from models 1–6 produced via all subset regression procedures (Table 5). The dotted line represents the line of the perfect fit. (N = 45; only those groups for which NUE, WS/CP, and MUN data were all available and have been included).

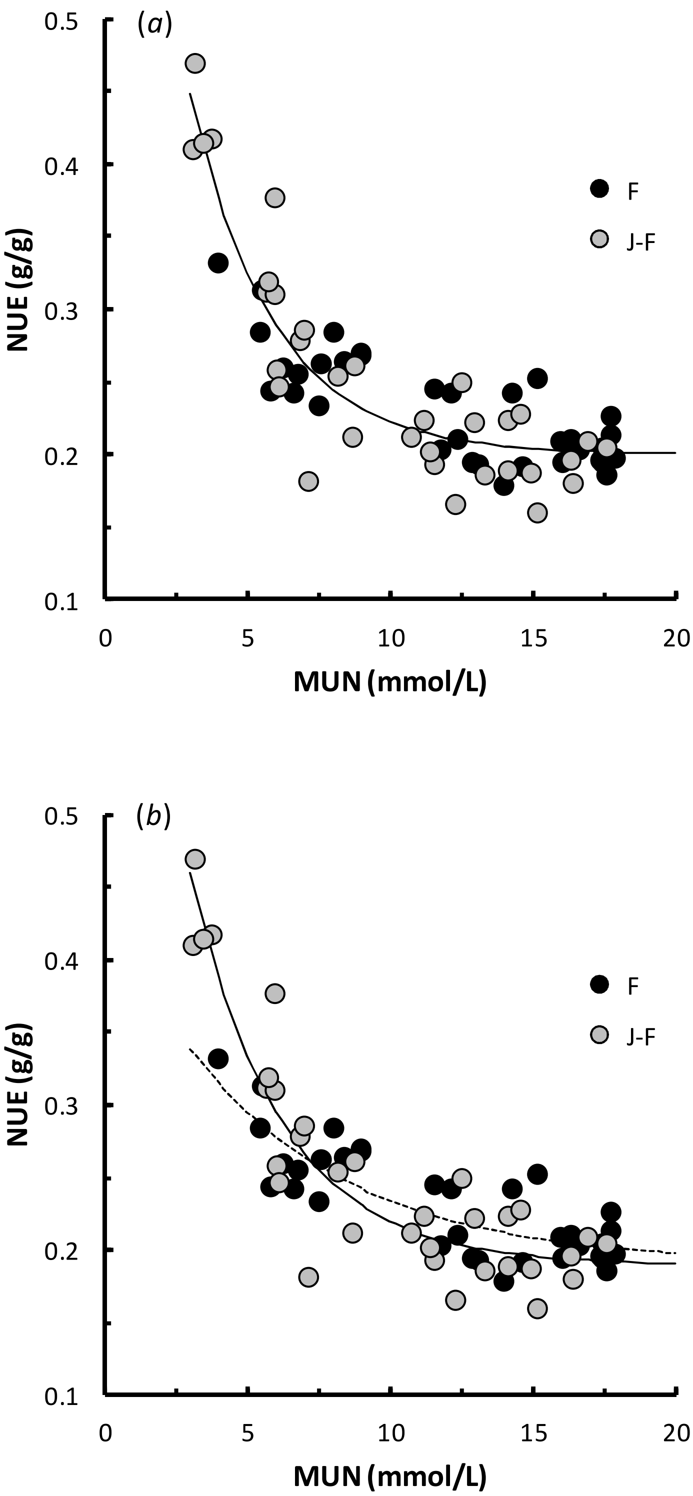

Figure 3. Asymptotic relationships between NUE and MUN for groups of ryegrass-fed Friesian (N = 35) and Jersey × Friesian (N = 33) cows: (**a**) shows the overall line of best fit (Model 10; Table 6) and (**b**) shows separate regression models for both breeds (Models 11 and 12; Table 6).

3.4. Model Evaluation

When evaluating these models with newly obtained data for both breeds, the MAE and RMSE were lesser when using the MUN-based model than the models based on WSC/CP (Table 7). This translated to standardised errors (MRAE and NRMSE) of approximately 11.5–18.5% when using the WSC/CP equations and approximately 7.5–13.5% when using the MUN model. Although the predicted and observed values of NUE were highly correlated when using the breed-specific linear equations based on WSC/CP, concordance was low, especially for Friesians (Table 7; Figure 4). Conversely, concordance was higher for both breeds when using the model based on MUN (Table 7; Figure 4).

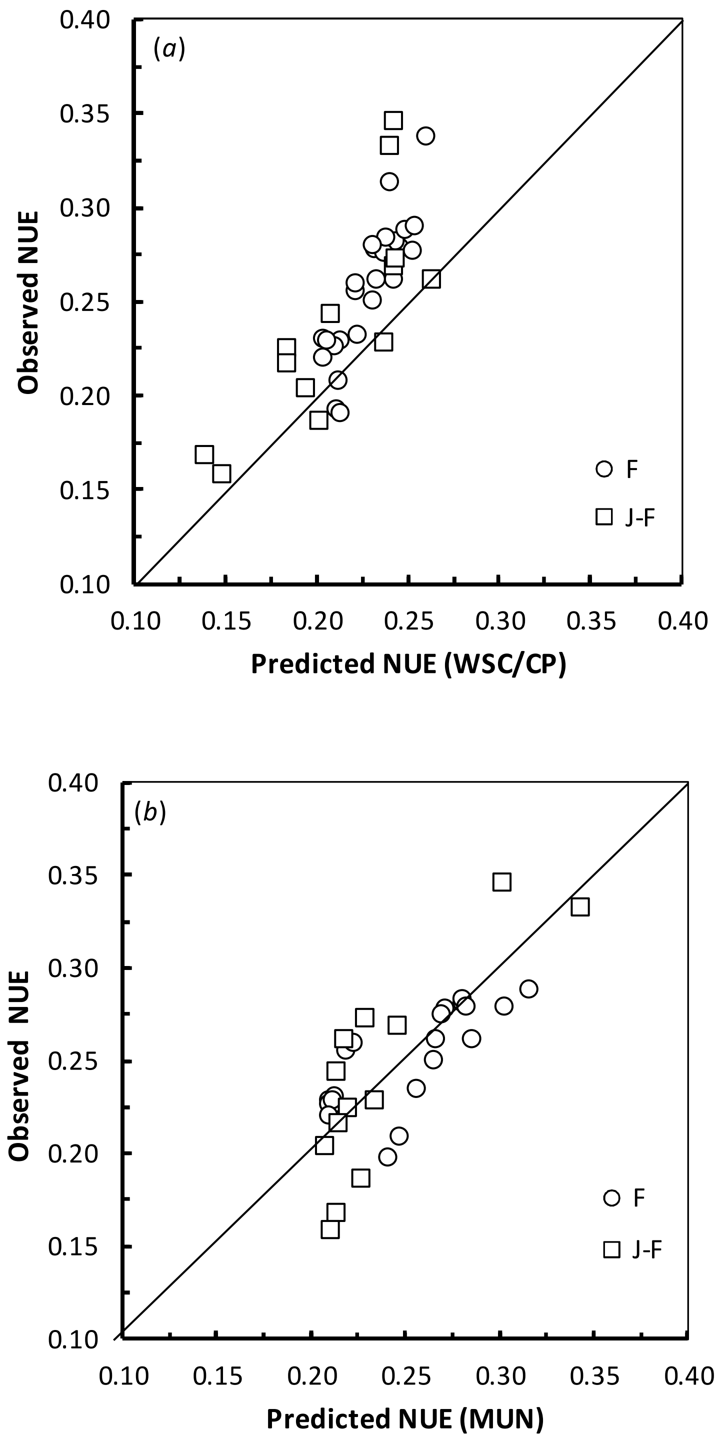

Figure 4. Correspondence of observed NUE values with those predicted using models based on (**a**) WSC/CP (Models 7 and 8; Table 6) and (**b**) MUN (Model 10; Table 6) for groups of Friesian (N = 27) and Jersey × Friesian (N = 13) cows not used in model development. The straight line indicates one-to-one correspondence between observed and predicted values.

Table 7. Measures of deviation between observed and predicted values of NUE for data obtained from studies not used in model development: mean absolute error (MAE), root mean square error (RMSE), mean relative absolute error (MRAE), and normalised root mean square error (NRMSE). Relationships between observed and predicted values of NUE are illustrated using Pearson's correlation coefficient and Lin's concordance coefficient. Friesians N = 27; Jersey × Friesian N = 13. Model numbers refer to the equations presented in Table 6.

Predictor	Models	Measure of Model Fit	F	J-F
WSC/CP	8 & 9	MAE	0.031	0.033
NUE = $a + b$(WSC/CP)		RMSE	0.035	0.045
		MRAE (%)	11.57	12.81
		NRMSE (%)	13.77	18.67
		Correlation	0.870	0.796
		Concordance	0.459	0.630
MUN	10	MAE	0.018	0.027
NUE = $a + bc^{MUN}$		RMSE	0.022	0.033
		MRAE (%)	7.74	12.26
		NRMSE (%)	8.91	13.64
		Correlation	0.748	0.811
		Concordance	0.729	0.764

4. Discussion

4.1. The Database, Rank Correlations, and Simple Linear Regression Models

The dataset covered a wide range of MUN values; some are high values, possibly due to dietary ryegrass that were fertilised with urea as part of the growth-promoting strategy. The average MUN concentration was slightly higher for Friesians than Jersey × Friesians (12.2 vs. 10.1 mmol/L, respectively). Similarly, previous reports [14,43] reported higher MUN concentrations in the milk of Holsteins in comparison with Jersey cows. In addition, Jonker et al. (1999) [44] suggested factors such as body weight, milk production, milk protein, and fat concentration, and N intake contribute to breeding differences in MUN concentrations.

According to the rank correlations of parameters with NUE for grazing Friesian and Jersey × Friesian dairy cows, many of the parameters had strong, significant rank correlations with NUE, indicating that they are potentially useful indicators for a simplistic assignment of NUE ranking. High absolute values for R^2 were found for dietary parameters (e.g., N, CP, ME/CP, and WSC/CP), animal-based parameters (e.g., NI, MUN), and some derived measures (e.g., MN/UN, UN/NI). Dietary parameters based on fibre content (ADF, NDF) were not strongly related to NUE. A similar weak correlation between NUE and dietary fibre content was shown in the study of Pacheco et al. [6]. Parameters based on dietary ME were not considered further in this study due to the low number of data points for Friesians ($n = 9$).

The high correlations between NUE and WSC/CP for dairy cows on the pasture-based systems have been described previously [6,45]. Edwards et al. [45] reported that increasing WSC concentration could correct the imbalance between the energy and protein supply in the rumen. This increase in WSC concentration would improve the capture of ruminal ammonia into microbial protein and influence the supply of absorbed amino acids for milk protein production [46]. Furthermore, ruminal carbohydrate digestibility and efficiency of ruminal N fermentation can only affect MUN indirectly through either an increase in milk N excretion, a decrease in N intake, or an increase in faecal N with a net result of reducing urinary N excretion [10].

However, a positive relationship between NUE and WSC is not always observed, for example, in cows offered a high CP diet [29]. Further, Pacheco et al. [6] and Edwards et al. [45] reported WSC/CP of 0.75 and 0.7, respectively, as the breakpoints above which NUE was affected. In the current study, most of the reported values for the WSC/CP were above the suggested breakpoint, ranging from 0.5 to 2.6 for Friesians and from 0.7 to 1.8 for the Jersey × Friesian cows, which may explain the significant relationship between NUE and WSC/CP in the current data set. Therefore, the linear regression models developed using WSC/CP (Models 8 and 9) should be treated with caution, as additional validation is required for cows fed pasture with WSC/CP below 0.7.

Of all individual animal factors, MUN showed consistently the highest R^2 values (>63%) for both breeds. This is in agreement with the study of Broderick and Clayton [8], who reported a strong relationship between the NUE and MUN (R^2 = 63%) for cows offered balanced rations under a confinement system. It is important to note that NUE was under-predicted when Broderick and Clayton's [8] model was evaluated using the current data set. This suggests that predictive models for NUE using MUN may only be of value for specific management systems (e.g., pasture vs. mixed rations) and/or specific breeds, such as Jersey × Friesian. Kauffman and St-Pierre [10] reported that breed had a significant effect on N intake because the Holstein cows had a higher DMI than the Jersey cows. The effect of breed on the UN–MUN relationship was considerable but fully explained by BW differences as a scale factor. Rodriguez et al. [14] reported a significant effect of breed on MUN, with milk from Holstein cows 40% higher in MUN than milk from Jersey cows. To the best of our knowledge, no published studies in the literature reported Jersey × Friesian differences in NUE and MUN levels. This is an area that requires more research.

The highest adjusted R^2 obtained was 83.9% for the derived measure MN/UN ratio. However, this is not surprising, as the calculation for NUE also has MN as the numerator and may not reflect a biological relationship. In addition, MN and UN are challenging to measure, particularly for a large number of cows under grazing conditions. Therefore, the MN/UN ratio was considered an inappropriate and impractical parameter to include in predictive models for NUE. Similarly, NI of grazing cows is difficult to measure and is also used in the calculation of NUE, and thus, it was not considered further.

4.2. All Subset Regression Analyses and Multiple Regression Models

Despite the ranking and predictive ability of the models, using multiple regression models did not improve the prediction of NUE, compared with the regressions using a single predictor. For example, the simple regression using MUN to predict NUE produced an adjusted R^2 of 77% and 66% for the Friesian and Jersey × Friesian, respectively. These adjusted R^2 are close to the value of 70% for Model 4, in which multiple parameters (MUN, WSC/CP, and breed) were used to predict NUE. This result implies there is no need to use multiple factor models to predict NUE of grazing Friesian or Jersey × Friesian dairy cows. Instead, models using a single parameter (e.g., MUN or WSC/CP) produced a similar predictive ability of NUE prediction, which is important for industry application.

4.3. Practical Parameters: The Relationships of NUE with WSC/CP and MUN

Using separate models (Models 8 and 9) for different breeds may be considered a valuable way of accounting for the variation between animals, mainly when dietary factors are used to predict NUE [7]. Although separate models are required for the two breeds in our study, further research would be required to examine whether separate models are required for all breeds. In New Zealand, where the significant dairy herds are limited to only a few breeds (Jerseys, Friesians, Holsteins, and their crosses), developing separate models for each breed might not be too onerous a task.

The asymptotes of all three NUE *v* MUN models (Models 10–12) are ≈0.2, which suggests there is some point (approximately MUN = 10 mmol/L) at which NUE becomes less responsive to any further increase in MUN. The corresponding NUE value of approximately 20% is in good agreement with the floor of 19% reported for dairy cows grazing fresh herbage [29]. MUN may not be useful for differentiating NUE at high levels of N intake [8,9], possibly because higher levels of N are being excreted in the urine rather than being used for milk protein production [5,47]. Therefore, the alternative biomarker should be explored to indicate NUE in high N intake conditions [48]. Further, Castillo et al. [47] reported that urine is the main route for N excretion when N intake of average yielding dairy cows exceeds 400 g N/cow/d. On the other hand, when N is limiting, the proportion of urea recycled back to the gut increases to ensure adequate N is available to meet the requirements for maintenance and production [49,50]. Thus, an N limiting diet results in a reduction in MUN and a corresponding increase in NUE. Nevertheless, the current results suggest that when efforts are being made to improve NUE, MUN is a valuable, easily obtained monitoring tool for NUE, particularly with MUN values below 10 mmol/L.

4.4. Model Evaluation

This observed higher concordance for both breeds when using the model based on MUN may support the previous suggestion that WSC/CP is a dietary measurement and does not take account of variation in N metabolism between and within breeds. In contrast, MUN may reflect the differences in N metabolism between breeds and dietary effects. In addition, the chemical composition of pasture is subjected to diurnal fluctuations [51,52], and therefore, that time of sampling may affect the accuracy of NUE predictions when using WSC/CP. Overall, the result indicates that MUN [Model 10] should be used to predict NUE for grazing dairy cows.

5. Conclusions

Milk urea nitrogen and dietary WSC/CP were the best and most practical parameters to predict NUE for grazing dairy cows. Using WSC/CP to predict NUE, separate models need to be used for different cow breeds. Overall, the model evaluation process indicated that the model using MUN as the independent variable resulted in predicted values of NUE that were closer to the observed NUE than did the models using WSC/CP. Additional validation of these models using other herds of dairy cows from the same and other geographic regions and of the same and other breeds is required before this can be used as a valid index to differentiate the NUE of all dairy cows under pasture-based feeding.

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