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# Modern Holstein-origin dairy cows within grassland-based systems partition more feed nitrogen into milk and excrete less in manure

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## ABSTRACT

The objective was to determine whether modern Holstein-origin dairy cows, when managed within grassland-based systems, partitioned more feed nitrogen (N) into milk and excreted less in manure, in comparison to an earlier population of Holstein-origin dairy cows. Data used were collated from total diet digestibility studies undertaken in Northern Ireland from 1990 to 2002 (old dataset, n = 538) and from 2005 to 2019 (new dataset, n = 476), respectively. An analysis of variance indicated that cows in the new dataset partitioned a significantly higher proportion of consumed N into milk and excreted a lower proportion in urine and total manure, compared to cows in the old dataset. A second analysis using the linear regression revealed that in comparison to the old dataset, the new dataset had a lower slope in the relationship between N intake and N excretion in urine or total manure, while a higher slope in the relationship between N intake and milk N output. A third analysis used the combined data from both datasets to examine if there was a relationship between experimental year and N utilization efficiency. Across the period from 1990 to 2019, urine N/N intake and manure N/N intake significantly decreased, while milk N/N intake increased. These results indicate that modern Holstein-origin dairy cows utilize consumed N more efficiently than earlier populations. Thus, N excretion is likely to be overestimated if models developed from the old data are used to predict N excretion for modern dairy herds. Therefore, the final part of analysis involved using the new dataset to develop prediction models for N excretion based on N intake and farm level data (milk yield, live weight and dietary N concentration). These updated models can be used to estimate N excretion from modern Holstein-origin dairy cows within grassland-based dairy systems.

**Keywords*:*** Grassland-based system, Holstein-origin cow, Manure nitrogen, Milk nitrogen, Prediction equation

**Abbreviations:**

AFBI, Agri-Food and Biosciences Institute; ANOVA, analysis of variance; CP, crude protein; DIM, days in milk; DN, diet nitrogen concentration; DM, dry matter; DMI, dry matter intake; ECMY, energy corrected milk yield; EU, European Union; FG, fresh grass; GS, grass silage; LW, live weight; ME, metabolizable energy; MS, maize silage; N, nitrogen; NDF, neutral detergent fiber; NI, nitrogen intake; RMSPE, root mean square prediction error; WCW, whole crop wheat silage;

## 1. Introduction

The loss of nitrogen (N) from livestock production systems can have a significant environmental impact (Tamminga, 1992; Yan *et al*., 2006). For example, N losses to waterways can cause aquatic eutrophication, N emissions as nitrous oxide can lead to stratospheric ozone depletion and to global warming, while ammonia deposition on sensitive ecosystems can result in terrestrial eutrophication and soil acidification (Asman *et al*., 1998; Hoekstra *et al*., 2020). While dairy cows have a large requirement for N, with dairy cow diets typically containing crude protein (CP) in a range between 160 and 180 g/kg dry matter (DM) (Webster, 2020), much of feed N consumed is in excess of what animals can utilize, and is excreted in feces and urine (Huhtanen *et al*., 2010; Powell *et al*., 2017).

Urea comprises between 50% and 90% of total N in urine of high-producing dairy cows, and this urea is rapidly converted to ammonia, which is lost by volatilization when feces and urine mix (Bussink and Oenema, 1998; Hristov *et al*., 2011). In Europe, approximately 75% of ammonia emitted to the atmosphere can be attributed to livestock production (Ding *et al*., 2020). Accurate predictions of the environmental impact of livestock production systems (for example, for estimating N volatilization, leaching, run-off, and emission), require N excretion from individual animals or groups of animals to be quantified with reasonable accuracy, and this is normally obtained from having an accurate estimate of N intakes and N utilization efficiency. A number of prediction models have been developed to predict N excretion in feces and urine from dairy cattle (e.g., Wilkerson *et al*., 1997; Yan *et al*., 2006; Reed *et al*., 2015).

The N utilization efficiency of dairy cows can be influenced by both dietary and animal factors, with diet quality (especially N concentration) and cow genetic merit likely to have a significant effect on the efficiency with which dietary N is converted into milk N (Ferris *et al*., 2018; O'Sullivan *et al*., 2019). During the last 20 years dairy cow genotypes have improved considerably due to sire selection programs in most counties now focusing on both functional traits (e.g., fertility, health) and production traits (e.g., higher yielding cows with the ability to partition a greater proportion of nutrients into milk and less into body tissues) (Ferris *et al.,* 2018; Derno *et al*., 2019). For example, the average annual milk production in the national dairy herd of Northern Ireland increased from 6,200 kg/yr in 2004 to 7,620 kg/yr in 2018 (Department of Agriculture, Environment and Rural Affair, 2018). These improvement in cow genetic merit requires dairy producers to offer cows higher quality diets so as to meet their higher nutrient requirements. However, this may pose a great challenge for dairy producers in the European Union (EU) countries, due to the implementation of the Nitrate Directive program in the EU in 2000s that restricts application rates of organic and inorganic N to agricultural lands, forcing the dairy industry to adopt balanced diets with reduced N input. These factors can obviously influence the N utilization efficiency of dairy cow production. However, there is little information available to systematically evaluate if modern Holstein-origin dairy cows, managed within grassland-based dairy systems, can utilize N more efficiently than earlier populations of Holstein-origin dairy cows. Therefore, the present study used the analysis of variance (ANOVA) and linear regression techniques to examine if the N utilization efficiency of dairy cows differed within two dairy cow datasets which were collated from total diet digestibility studies undertake at the Agri-Food and Biosciences Institute (AFBI) of Northern Ireland from 1990 to 2002, and from 2005 to 2019, respectively. The latter dataset was also used to develop prediction equations for N excretion for modern dairy cow production. The division of the year gap between the two datasets was due to the implementation of the EU's Nitrate Directive program in Northern Ireland in 2005-

2006. This program restricts application rates of N fertilizers to agricultural lands that consequently forces the dairy industry to reduce N input for dairy production.

# 2. Materials and Methods

## *2.1. Animal, Diet and Digestibility Measurement*

Two N utilization datasets for lactating dairy cows were used in the present study, data within each having been collated from total diet digestibility studies undertaken at AFBI in Northern Ireland. The first dataset comprised data from experiments undertaken between 1990 and 2002 (n = 538), while the second dataset comprised data from experiments undertaken between 2005 and 2019 (n = 476). Hereafter, these datasets are referred to as the 'old dataset' and the 'new dataset', respectively. The new dataset was also used to develop prediction equations for N excretion for modern dairy cow production. The old dataset represents data collected prior to the implementation of the EU's Nitrate Directive in Northern Ireland in the form of a Nitrates Action Program in 2005-2006.

The information on numbers of experiments, treatments, and cows, on cow genotypes, and forage types offered within each of the two datasets are presented in Table 1. Data on milk production, feed intake, N intake and outputs, and N utilization efficiency, within the 2 datasets are presented in Table 2. Before commencing the digestibility trials, all cows were housed in free-stall cubicle accommodation and offered experimental diets *ad libitum* for at least 20 d. Thereafter, all cows were transferred to a metabolism unit for a further 8 d. During this time feed intake was recorded daily, while samples of forages and concentrates offered were taken daily and analyzed for chemical composition. Feces and urine were collected separately and sampled daily during the final 6 d in the metabolism unit to allow total ration digestibility to be determined. Details of feces and urine collection, feed sampling, and methods used for analysis of feed, feces and urine samples were as described by Yan *et al*. (2006). Milk yields were recorded daily with milk samples taken during both morning (starting at 0500 h) and afternoon (starting at 1630 h) milking during the 8 d in metabolism units. Fat, protein and lactose concentrations of milk samples were analyzed using the methods described by Yan *et al*. (2006). Live weight (LW) was recorded on the first and last d in the metabolism unit. Animals had free access to water throughout the whole experimental period.

### *2.2. Statistical Analysis*

Data analysis was conducted using Genstat 19th edition (VSN International, 2017). The two datasets (e.g., feed intake, milk production, N intake and output, and N utilization efficiency) were firstly compared using ANOVA, with the effects of animal [LW, milk yield, parity, days in milk (DIM), days in pregnancy] and dietary [forage proportion and concentrations of neutral detergent fiber (NDF), CP and metabolizable energy (ME)] factors removed, where appropriate. Linear regression analysis was then used to related total N intake, to N output in feces, urine or manure, with the objective to evaluate if there was significant difference in the slopes (with a common intercept) between the two datasets (old data vs. new data), or if there was any significant difference in the intercepts (with a common slope). The relationship between each response variable and each explanatory variable was fitted as a linear mixed model using the residual maximum likelihood (REML) commands. Diets and animals within experiments were fitted as random effects in all models, and the explanatory variable was as the fixed effect. Additional combinations of covariates, when appropriate, were also fitted as supplementary random effects for evaluation of N utilization efficiencies, which included milk yield, parity, DIM, days in pregnancy, dietary forage proportion, and dietary contents of NDF, CP and ME. The significance or otherwise of fixed effects was assessed by comparing a Wald statistic against the appropriate F-distribution. If any of additional fixed effects was not significant ($P > 0.05$), then it was removed from the analysis and the model was refitted. Several different models were fitted to each pair of response/explanatory variables in turn. First, a single line was fitted for all two datasets, and then two linear relationships using the two datasets (old vs. new datasets) were developed to compare the two slopes (with a common intercept) or the two intercepts (with a common slope). For the latter two models, pair-wise differences between different intercepts or slopes were also calculated if the main effect was significant using the Fisher's least significant difference test. Finally, an assessment of the goodness-of-fit of each model was made by calculating a pseudo $R^2$ (calculated in each case as the square of the correlation of the fitted valued from the model with the observed values for the response variable). A third analysis involved examining if there was a linear relationship between experimental year and N partitioning rates for milk production and manure N excretion, using the combined data within the old and new datasets. Random effects were taken into account for each model, including experiment and animal (LW, milk yield, parity, DIM, days in pregnancy) and dietary (forage proportion and concentrations of NDF, CP and ME) factors.

Since the above comparisons demonstrated that the new dataset had a significantly higher N utilization efficiency than the old dataset, the new dataset was then used to develop a range of new models for predicting N excretion from 'modern' dairy herds. These new models (linear and multiple regression models) were developed, using the REML variance components analysis, to predict N excretion in feces, urine or total manure using N intake or a combination of LW, milk yield and dietary N concentration as explanatory variables. Random factors, including experiment, trial year, forage type, breed, parity and DIM, were fitted into each model with the objective of removing the effects of these random factors from each relationship. These new equations were evaluated through an internal validation exercise, by dividing the whole new dataset (n = 476) into two sub-datasets, i.e., two-thirds of data (n = 317) vs. one-third of data (n = 159). The selection was based on individual treatments/periods within each study, which ensures that each sub-dataset had a similar presentation of data variations as the whole dataset. The two-thirds of data were used to develop similar prediction equations to those developed using the whole dataset. These new prediction equations were then evaluated using the one-third of data. Prediction accuracy of relationships was examined using the root mean square prediction error (RMSPE), which is defined below (Equation a):

$$RMSPE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(P_i - A_i)^2} \quad [a]$$

Where $P_i$ or $A_i$ is the predicted or actual N output; $n$ is the number of pairs of values of $P_i$ and $A_i$ compared.

## 3. Results

### *3.1. Comparison of Cow Performance and N Utilization Data between the Old and New Datasets Using ANOVA*

In comparison to the old dataset, cows in the new dataset had higher milk yield, energy-corrected milk yield (ECMY) and DIM, but lower LW ($P < 0.001$; Table 2). Daily forage DM intake (DMI), concentrate DMI and total DMI were 0.7 kg, 1.1 kg and 1.8 kg higher ($P < 0.001$), respectively, in the new compared to the old dataset, but diets offered in the new dataset had a lower forage proportion ($P = 0.015$). Diets in the old dataset had a mean CP concentration of 0.011 kg/kg DM higher than those in the new dataset ($P < 0.001$), while mean diet ME concentration was identical between the two datasets.

Cows in the new dataset had a greater N intake ($P = 0.015$), and consequently higher ($P < 0.001$) feces N output, milk N output and retained N than those in the old dataset, while those in the old dataset had a higher ($P < 0.001$) urine N output and manure N output. Nitrogen losses from urine and manure, when expressed as a proportion of N consumed, were lower ($P < 0.001$) for cows in the new than the old dataset, but feces N, milk N and retained N as a proportion of N intake were higher ($P < 0.001$) for those in the new dataset.

*3.2. Regression Analysis of N Utilization Data between the Old and New Datasets*

The linear regression technique was used to determine if there were differences in N utilization efficiency between the old and new dataset, through the comparison of slopes (with a common intercept) or intercepts (with a common slope) in each set of the linear relationship between N output and N intake. The results for comparison of slopes (with a common intercept) are presented in Table 3 and Figure 2. Feces N, urine N, manure N, milk N and retained N were each positively and significantly ($P < 0.05$) related to N intake, with $R^2$ values ranging from 0.517 to 0.905 (Eq. [1a] to [5b]). With a common intercept, in comparison to the old dataset, the new dataset had a greater slope in the relationship of N intake with feces N ([1a] vs. [1b], $P = 0.037$), milk N ([4a] vs. [4b], $P < 0.001$) and retained N ([5a] vs. [5b], $P = 0.009$), but a lower slope in relationship of N intake with urine N ([2a] vs. [2b], $P <0.001$) and manure N ([3a] vs. [3b], $P < 0.001$). A similar result for comparison of intercepts (with a common slope) was also obtained (Table 4). With a common slope, intercepts derived from relationships of N intake with feces N ([6a] vs. [6b]), milk N ([9a] vs. [9b], $P = 0.011$) and retained N ([10a] vs. [10b], $P = 0.035$) were bigger in the new than old dataset, while the new dataset had a lower intercept in the relationship with urine N ([7a] vs. [7b], $P < 0.001$) and manure N ([8a] vs. [8b], $P < 0.001$).

*3.3. Relationships between Experimental Year and N Utilization Using the Combined Data*

The third evaluation was undertaken to examine if there was any relationship between experimental year and N utilization efficiency using the combined data from both old and new datasets. The results are presented in Table 5. The result revealed a negative relationship between experimental year and both urine N/N intake and manure N/N intake, and a positive relationship with milk N/N intake.

*3.4. Prediction Equations for N Excretion Developed Using the New Dataset*

Since the above evaluation indicates that 'modern cows' in the new dataset can utilize diet N more efficiently than cows in the old dataset, a range of updated prediction equations for N excretion for modern dairy production were developed using the new dataset (Table 6). The relationships between N excretion and N intake are also presented in Fig. 1. All relationships were significant ($P < 0.001$), and each predictor had a significant effect on the relationship ($P < 0.001$). Nitrogen intake is a good predictor of N excretion in urine and manure ($R^2 = 0.783$ and 0.833, respectively), although the $R^2$ value (0.684) for prediction of feces N output is relatively low. As N intake data are not always available, especially in commercial farms, farm-level data (ECMY, LW and diet N concentration) were also used to develop prediction equations. The $R^2$ values were 0.774 and 0.779, respectively, for prediction of N excretion in urine and total manure, although the $R^2$ value for prediction of feces N output is relatively low ($R^2 = 0.593$).

These updated equations were evaluated through an internal validation exercise (Table 7). All equations produced a mean predicted value that is close to the mean actual data in the prediction of N excretions in feces, urine and total manure. All predictions had a relatively small RMSPE. In addition, farm level data (ECMY, LW and diet N concentration) can be used to predict feces N and urine N outputs with a similar accuracy to those predicted using N intake, in terms of RSMPE and SE values, although prediction of manure N output had marginally higher RSMPE and SE values when predicted using farm level data.

## 4. Discussion

The present study was designed to evaluate the effects of dietary N inputs, and genetic improvements within the Holstein dairy cow population, on N utilization efficiency for milk production and N excretion rate in manure. Within the EU, pressure to improve N utilization efficiency has been driven in part by the EU Nitrates Directive which was designed to reduce N losses of agricultural origin to waterways (EU, 1991), as well as concerns about global warming, and the impact of ammonia on sensitive habitats. The two datasets used in the present study were obtained from studies undertaken at AFBI in Northern Ireland, and involved dairy cows of the Holstein breed (including Holstein crossbreds), offered predominantly grass silage based diets. However, grassland-based systems in Northern Ireland have much in common with systems adopted in many other grassland regions of the world, including western parts of the United Kingdom, Republic of Ireland and much of Northern Europe. In addition, the AFBI herd is bred entirely by artificial insemination, using high genetic merit sires sourced globally, and as a result is genetically similar to many high producing Holstein herds throughout the world. Thus the outcomes of the present study has applicability beyond Northern Ireland.

*4.1. Nitrogen Utilization Efficiency*

The present study indicates that modern dairy cows utilize feed N more efficiently than previous dairy cow populations (over 15 years ago). In comparison with the old dataset, cows in the new dataset utilized a higher proportion of N intake for milk production, and excreted a lower proportion of N intake in urine and total manure. A linear regression between experimental year and N utilization efficiency data involving the combined old and new datasets demonstrated a significant reduction in the ratios of urine N/N intake, and manure N/N intake, and a significant increase in milk N/N intake over the last two decades. These results imply that, with lower diet N inputs, modern dairy herds can maintain a similar milk production and excrete less N in manure, when compared to those over 15 years ago. In addition, it is worth noting that cows in the new dataset had a considerably lower proportion of urine N over N intake. The reduction in urinary N excretion is likely to help reduce ammonia loss to the environment, with potentially beneficial effects on air quality and biodiversity in sensitive habitats.

Many dietary, animal and management factors can influence N utilization efficiency of dairy cows (ARC, 1980). Perhaps, the most important factor is to feed dairy cows balanced diets which synchronize the supply of degradable N and fermentable energy to optimize rumen microbial activity and milk production. The oversupply of degradable N can cause the excessive ammonia in the rumen to be absorbed into bloodstream and excreted in urine as urea (Burgos *et al*., 2010). In the present study, the higher N utilization efficiency derived from the new vs. old dataset could be attributed to lower dietary CP concentrations in the new dataset (0.174 vs. 0.183 kg/kg DM, $P < 0.001$), because dietary ME concentration in the two datasets was identical, although the new dataset had a slightly lower dietary forage proportion (0.554 vs. 0.579 kg/kg DM, $P = 0.006$). The statistical analysis of the present two datasets found that the new dataset had lower ratios of urine N and manure N over N intake, although fecal N/N intake was higher in the new dataset. The linear regression analysis using the combined data of the present new and old datasets also found a similar result (Fig. 3).

Increasing dietary CP concentrations significantly increased N excretion rates in urine and total manure but decreased fecal N output rate ($P < 0.001$). Although there is no comparable publication using data collated from a range individual total diet studies undertaken at different periods of years, there are a range of individual studies of dairy cows which obtained similar results to the present study. For example, Broderick (2003) found a reduced urine N (from 0.362 to 0.238 g/g) but increased fecal N (from 0.296 to 0.403 g/g) as proportion of N intake in lactating dairy cows offered diets containing dietary CP varied from 0.135 to 0.194 kg/kg DM. Hristov *et al*. (2004) reported that increased dietary CP concentration resulted in decreased efficiency of conversion of dietary N into milk protein and less efficient use of ruminal ammonia N for milk protein syntheses, with excess largely lost through urinary N excretion. Increasing dietary CP concentrations were found to increase dilution of metabolic fecal N, and increase N digestibility, and also increase urinary N excretion (Marini and Van Amburgh, 2005). In addition, reduced dietary N/ME and CP concentration have been reported to improve N utilization efficiency with less N excreted in urine of dry cows (Stergiadis *et al*., 2015a). The reduction of N excretion in urine implies less ammonia emissions from dairy production systems, as urinary urea can be rapidly hydrolyzed to ammonia by the urease enzyme in less than 24 h in grazing (Petersen *et al*., 1998) and confined animals (James *et al*., 1999). Frank *et al*. (2002) found, on average, a 2/3 decrease in ammonia release to air from manure of dairy cows offered diets containing CP of 0.140 vs. 0.190 kg/kg DM without significant effect on milk production. These findings, together with the present result, indicate that manipulating dietary CP concentration could be an effective strategy to improve N utilization efficiency and reduce N excretion and ammonia emissions in dairy cow production.

The increase in N utilization efficiency observed with the modern dairy cows in the new dataset may also be due to the continuous improvement in cow genetic merit over time. Indeed, cow genetic merit (expressed as £Profitable Lifetime Index, 2018 base year) of Holstein cows in AFBI dairy herd, from which dairy cows used in experiments of the present study were selected, improved by £23.3 per year from 1993 to 2017 (Fig 4). Profitable Lifetime Index, a composite financial index used within the UK, includes milk production and a number of other functional traits including health, fertility and longevity. Selecting sires on the basis of £ Profitable Lifetime Index has also improved the milk production potential of the herd, resulting in cows with higher nutrient requirements to meet their greater energy demand for milk production. Increasing the level of feeding can increase the rumen outflow rate, and leave less time available for rumen microbial activity, thus reducing protein degradability in the rumen and consequently N excretion in urine. Indeed, in a study to evaluate the effect of cow genetic merit on the production efficiency, Ferris *et al*. (1999) found that high merit cows had higher DM intake and milk production, but lower urine N output as a proportion of N intake, when compared with low merit cows. Yan *et al*. (2006), in a meta-analysis of a large digestibility dataset, reported a reduced ratio of manure N/N intake with increasing milk yield from <15, 15-30 to >30 kg/d. Cheng *et al*. (2014) reported a positive relationship between N utilization efficiency and cow's genetic merits when fed with freshly-cut perennial ryegrass. On the other hand, high genetic merit cows were found to have the ability to partition more nutrients into milk and less into body tissue than medium or low genetic merit cows (Agnew and Yan, 2000; Mehtiö *et al*., 2018). Gordon *et al*. (1995) demonstrated that high genetic merit cows produced 6.60 and 8.25 kg/d more milk, and partitioned 13% and 8% more consumed N into milk, respectively, when compared with medium and low genetic merit cows. These results indicate that high genetic merit cows utilize feed N for milk production more efficiently than lower genetic merit cows.

Consequently, modern dairy cows can excrete less N in feces and urine, per kg of standard milk.

*4.2. Prediction Equations for N excretion*

The present study revealed that modern dairy cows had a higher N utilization efficiency than previous populations over 15 years ago. Thus using equations developed using data from studies undertaken over 15 years ago may over-predict N excretions in feces and urine for modern dairy cows. Therefore, two sets of updated prediction equations for fecal N, urinary N and manure N were developed using the new dataset in the present study. One set of equations is based on N intake and the other based on farm level data (LW, milk yield and diet N concentration). Nitrogen intake has been found to be a better predictor of urine N (Reed et al., 2015) and manure N output (Yan *et al*., 2006) than farm level data (e.g., LW or LW and milk yield) in both dairy cows and beef cattle (Dong *et al*., 2014; Jiao *et al*., 2014; Reed *et al*., 2015). In the present study, using N intake as a single predictor for fecal N, urine N and manure N output produced responses with relatively high $R^2$ values (0.684, 0.783 and 0.833, respectively). These values are comparable to those in young Holstein steer and heifer offered grass silage (0.75, 0.73 and 0.86, respectively; Jiao *et al*., 2014), but higher than those in non-pregnant cows offered fresh grass (0.50, 0.61 and 0.60, respectively; Stergiadis *et al*., 2015b), and that (0.78) of relationship between N intake and manure N output (Kebreab *et al*., 2001) using a small dataset of lactating dairy cows. Since information on N intake is not always available, especially on commercial farms, a range of prediction equations using farm level data (LW, milk yield and diet N concentration) were also developed in the present study. Although the $R^2$ value (0.593) for prediction of feces N output was relatively low, the $R^2$ values for prediction of urine N (0.774) and manure N (0.779) are comparable to those derived in the current study using N intake as the predictor. The present internal validation also demonstrated that using these farm level data could produce a relatively accurate prediction of N excretion in feces, urine and total manure, when compared with those predicted using N intake. These equations provide a useful tool to estimate N excretion in feces and urine from Holstein-origin cows in commercial grassland-based dairy systems.

## 5. Conclusion

The present study showed that the modern Holstein-origin dairy cows managed within grassland-based systems utilized consumed diet N more efficiently, partitioning more consumed N into milk and less into urine and total manure, than earlier Holstein populations. The increase in N utilization efficiency not only improves the economical return to dairy producers, but also reduces N losses to the environment as nitrates, ammonia and nitrous oxide. In addition, the present study developed a range of prediction equations for manure N excretion using data collated from modern dairy cows, which provide a useful tool for the Holstein-origin dairy producers to mitigate N excretion under grassland-based farming conditions.

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## Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

## References


Agricultural and Food Research Council (AFRC), 1993. Energy and protein requirements of ruminants: an advisory manual pro-posed by the AFRC technical committee on responses to nutrients. CAB International, Wallingford, UK.

Agnew, R.E., Yan, T., 2000. Impact of recent research on energy feeding systems for dairy cattle. Livest. Prod. Sci. 66, 197-215. https://doi.org/10.1016/S0301-6226(00)00161-5.

Asman, W.A.H., Sutton, M.A., Schjørring, J.K., 1998. Ammonia: emission, atmospheric transport and deposition. New Phytol. 139, 27–48. https://doi.org/10.1046/j.1469-8137.1998.00180.x.

Broderick, G.A., 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86, 1370-1381. https://doi.org/10.3168/jds.S0022-0302(03)73721-7.

Bussink, D.W., Oenema, O., 1998. Ammonia volatilisation from dairy farming systems in temperate areas: a review. Nutr. Cycl. Agroecosys. 51, 19-33. https://doi.org/10.1023/A:1009747109538.

Burgos, S.A., Embertson, N.M., Zhao, Y., Mitloehner, F.M., DePeters, E.J., Fadel, J.G., 2010. Prediction of ammonia emission from dairy cattle manure based on milk urea nitrogen: Relation of milk urea nitrogen to ammonia emissions. J. Dairy Sci. 93, 2377-2386. https://doi.org/10.3168/jds.2009-2415.

Cheng, L., Woodward, S.L., Dewhurst, R.J., Zhou, H., Edwards, G.R., 2014. Nitrogen partitioning, energy use efficiency and isotopic fractionation measurements from cows differing in genetic merit fed low-quality pasture in late lactation. Anim. Prod. Sci. 54, 1651-1656. https://doi.org/10.1071/AN14171.

Department of Agriculture, Environment and Rural Affair, 2018. Statistical review of Northern Ireland agriculture. Report of the policy, economics and statistics division, Department of Agriculture, Environment and Rural Affairs, Belfast, Northern Ireland. Accessed Sept. 9, 2019. https://www.daera-ni.gov.uk/sites/default/files/publications/daera/stats-review-2018-final.pdf.

Derno, M., Nürnberg, G., Kuhla, B., 2019. Characterizing the metabotype and its persistency in lactating Holstein cows: An approach toward metabolic efficiency measures. J. Dairy Sci. 102, 6559-6570. https://doi.org/10.3168/jds.2019-16274.

Ding, L., Li, Q., Wang, C., Zhang, G., Jiang, R., Yu, L., Zheng, W., Gao, R., Ma, W., Zhang, S., Shi, Z., 2020. Determination of the mass transfer coefficient of ammonia emissions from dairy open lots using a scale model. Biosyst. Eng. 190, 145-156. https://doi.org/10.1016/j.biosystemseng.2019.12.008.

Dong, R.L., Zhao, G.Y., Chai, L.L., Beauchemin, K.A., 2014. Prediction of urinary and fecal nitrogen excretion by beef cattle. J. Anim. Sci. 92, 4669-4681. https://doi.org/10.2527/jas.2014-8000.

EU, 1991. Council Directive of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources (91/676/EEC). European Union. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1991:375:0001:0008:EN:PDF.

Ferris, C.P., Gordon, F.J., Patterson, D.C., Porter, M.G., Yan, T., 1999. The effect of genetic merit and concentrate proportion in the diet on nutrient utilization by lactating dairy cows. J. Agric. Sci. 132, 483-490. https://doi.org/10.1017/S0021859699006553.

Ferris, C.P., Purcell, P.J., Gordon, A.W., Larsen, T., Vestergaard, M., 2018. Performance of Holstein and Swedish-Red× Jersey/Holstein crossbred dairy cows within low-and medium-concentrate grassland-based systems. J. Dairy Sci. 101, 7258-7273. https://doi.org/10.3168/jds.2017-14107.

Frank, B., Person, M., Gustafsson G., 2002. Feeding dairy cows for decreased ammonia emission. Livest. Prod. Sci. 76, 171-179. https://doi.org/10.1016/S0301-6226(02)00021-0.

Gordon, F.J., Patterson, D.C., Yan, T., Porter, M.G., Mayne, C.S., Unsworth, E.F., 1995. The influence of genetic index for milk production on the response to complete diet feeding and the utilization of energy and nitrogen. Anim. Sci. 61, 199-210. https://doi.org/10.1017/S1357729800013722.

Hoekstra, N.J., Schulte, R.P.O., Forrestal, P.J., Hennessy, D., Krol, D.J., Lanigan, G.J., Müller, C., Shalloo, L., Wall, D.P., Richards, K.G., 2020. Scenarios to limit environmental nitrogen losses from dairy expansion. Sci. Total Environ. 707, 134606. https://doi.org/10.1016/j.scitotenv.2019.134606.

Hristov, A.N., Hanigan, M., Cole, A., Todd, R., McAllister, T.A., Ndegwa, P.M., Rotz, A., 2011. Ammonia emissions from dairy farms and beef feedlots: A review. Can. J. Anim. Sci. 91, 1-35. https://doi.org/10.4141/CJAS10034.

Hristov, A.N., Etter, R.P., Ropp, J.K., Grandeen, K.L., 2004. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. J. Anim. Sci. 82, 3219-3229. https://doi.org/10.2527/2004.82113219x.

Huhtanen, P., Ahvenjärvi, S., Broderick, G.A., Reynal, S.M., Shingfield, K.J., 2010. Quantifying ruminal digestion of organic matter and neutral detergent fiber using the omasal sampling technique in cattle - A meta-analysis. J. Dairy Sci. 93, 3203-3215. https://doi.org/10.3168/jds.2009-2988.

James, T., Meyer, D., Esparza, E., Depeters, E.J., Perez-Monti, H., 1999. Effects of dietary nitrogen manipulation on ammonia volatilization from manure from Holstein heifers. J. Dairy Sci. 82, 2430-2439. https://doi.org/10.3168/jds.S0022-0302(99)75494-9.

Jiao, H.P., Yan, T., McDowell, D.A., 2014. Prediction of manure nitrogen and organic matter excretion for young Holstein cattle fed on grass silage-based diets. J. Anim. Sci. 92, 3042-3052. https://doi.org/10.2527/jas.2013-7552.

Kebreab, E.J., France, J., Beever, D.E., Castillo, A.R., 2001. Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. Nutr. Cycl. Agroecosyst. 60, 275-285. https://doi.org/10.1023/A:1012668109662.

Marini, J.C., Van Amburgh, M.E., 2005. Partition of nitrogen excretion in urine and the feces of Holstein replacement heifers. J. Dairy Sci. 88, 1778-1784. https://doi.org/10.3168/jds.S0022-0302(05)72852-6.

Mehtiö, T., Negussie, E., Mäntysaari, P., Mäntysaari, E.A., Lidauer, M.H., 2018. Genetic background in partitioning of metabolizable energy efficiency in dairy cows. J. Dairy Sci. 101, 4268-4278. https://doi.org/10.3168/jds.2017-13936.

O'Sullivan, M., Horan, B., Pierce, K.M., McParland, S., O'Sullivan, K., Buckley, F., 2019. Milk production of Holstein-Friesian cows of divergent Economic Breeding Index evaluated under seasonal pasture-based management. J. Dairy Sci. 102, 2560-2577. https://doi.org/10.3168/jds.2019-16371.

Petersen, S.O., Sommer, S.G., Aaes, O., Soegaard, K., 1998. Ammonia losses from urine and dung of grazing cattle: Effect of N intake. Atmos. Environ. 32, 295-300. https://doi.org/10.1016/S1352-2310(97)00043-5.

Powell, J.M., Barros, T., Danes, M., Aguerre, M., Wattiaux, M., Reed, K., 2017. Nitrogen use efficiencies to grow, feed, and recycle manure from the major diet components fed to dairy cows in the USA. Agr. Ecosyst. Environ. 239, 274-282. https://doi.org/10.1016/j.agee.2017.01.023.

Reed, K.F., Moraes, L.E., Casper, D.P., Kebreab, E., 2015. Predicting nitrogen excretion from cattle. J. Dairy Sci. 98, 3025-3035. https://doi.org/10.3168/jds.2014-8397.

Stergiadis, S., Allen, M., Chen, X.J., Wills, D., Yan, T., 2015a. Prediction of nutrient digestibility and energy concentrations in fresh grass using nutrient composition. J. Dairy Sci. 98, 3257-3273. https://doi.org/10.3168/jds.2014-8587.

Stergiadis, S., Chen, X.J., Allen, M., Wills, D., Yan, T., 2015b. Evaluating nitrogen utilization efficiency of nonpregnant dry cows offered solely fresh cut grass at maintenance levels. J. Anim. Sci. 93, 709-720. http://dx.doi.org/10.2527/jas.2014-8197.

Tamminga, S., 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75:345-357. https://doi.org/10.3168/jds.S0022-0302(92)77770-4.

VSN International, 2017. GenStat for Windows, 19th ed. VSN International, Hemel Hempstead, UK. Genstat.co.uk.

Webster, J., 2020. Understanding the Dairy Cow, third ed. Wiley-Blackwell, Hoboken, New Jersey.

Wilkerson, V.A., Mertens, D.R., Casper, D.P., 1997. Prediction of excretion of manure and nitrogen by Holstein dairy cattle. J. Dairy Sci. 80, 3193-3204. https://doi.org/10.3168/jds.S0022-0302(97)76292-1.

Yan, T., Frost, J.P., Agnew, R.E., Binnie, R.C., Mayne, C.S., 2006. Relationships among manure nitrogen output and dietary and animal factors in lactating dairy cows. J. Dairy Sci. 89, 3981–3991. https://doi.org/10.3168/jds.S0022-0302 (06)72170-1.

**Figures:**

**Figure 1.** Relationships between N intake and N excretion using data of dairy cows collated from experiments undertaken at AFBI from 1990 to 2002 (old dataset, A) and from 2005 to 2019 (new dataset, B)

**Figure 2.** The comparison of N utilization efficiencies of dairy cows using data obtained between 1990-2002 (old dataset, dashed line) and 2005-2019 (new dataset, solid line) and the linear regression of N intake against N excretion in feces (A), urine (B) and total manure (C)

**Figure 3.** The relationships between diet crude protein (CP) concentration and N excretion ratios in feces (dashed line) and urine (solid line) using the combined data of old and new datasets in the present study

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**Tables:**

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**Table 1.** Information on experiment, treatment, cow breed and forage types in the old and new datasets of dairy cows used in the present study

| | Old dataset | New dataset |
|---|---|---|
| Years of experiments | 1990-2002 | 2005-2019 |
| Number of experiments | 25 | 14 |
| Number of treatments | 134 | 74 |
| Number of individual cow data | 538 | 476 |
| Cow breeds | | |
| Holstein-Friesian | 509 | 357 |
| Others[1] | 29 | 119 |
| Forage types[2] | GS, FG | GS, MS, WCW |

[1] Including Holstein crossbreds, Norwegian and Swedish Red.

[2] GS = grass silage, FG = fresh grass, MS = maize silage, WCW = whole crop wheat silage

**Table 2**
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**Table 2.** The ANOVA comparison of AFBI dairy cow digestibility variables using data obtained between 1990 and 2002 (old dataset) and 2005-2019 (new dataset)

| | Old dataset | New dataset | SED[1] | *P*-value |
|---|---|---|---|---|
| Number of cows | 538 | 476 | - | - |
| Animal data | | | | |
| Lactation number | 2.9 | 2.5 | 0.11 | <0.001 |
| Days in milk | 154 | 170 | 4.7 | <0.001 |
| Live weight, kg | 565 | 550 | 4.5 | <0.001 |
| Milk yield, kg/d | 21.3 | 23.6 | 0.46 | <0.001 |
| Energy-corrected milk yield, kg/d | 21.7 | 24.0 | 0.44 | <0.001 |
| Feed intake and composition[2] | | | | |
| Forage DMI, kg/d | 9.4 | 10.0 | 0.19 | <0.001 |
| Concentrate DMI, kg/d | 7.1 | 8.2 | 0.22 | <0.001 |
| Total DMI, kg/d | 16.4 | 18.2 | 0.20 | <0.001 |
| Forage proportion, kg/kg DM | 0.585 | 0.554 | 0.0112 | 0.006 |
| Diet CP concentration, kg/kg DM | 0.183 | 0.174 | 0.0017 | <0.001 |
| Diet ME concentration, MJ/kg DM | 12.1 | 12.1 | 0.06 | 0.96 |
| N intake and output, g/d | | | | |
| N intake | 484 | 506 | 8.0 | 0.006 |
| Feces N output | 141 | 159 | 2.3 | <0.001 |
| Urine N output | 208 | 178 | 4.4 | <0.001 |
| Manure N output | 349 | 337 | 6.0 | 0.045 |
| Milk N output | 108 | 127 | 2.3 | <0.001 |
| Retained N | 27 | 42 | 2.6 | <0.001 |
| N utilization efficiency | | | | |
| Feces N/N intake | 0.296 | 0.321 | 0.0034 | <0.001 |
| Urine N/N intake | 0.428 | 0.348 | 0.0051 | <0.001 |
| Manure N/N intake | 0.723 | 0.669 | 0.0047 | <0.001 |
| Milk N/N intake | 0.226 | 0.252 | 0.0035 | <0.001 |
| Retained N/N intake | 0.050 | 0.079 | 0.0051 | <0.001 |

[1]Standard error of the difference.

[2]DMI = dry matter intake, DM = dry matter, CP = crude protein, ME = metabolizable energy

Table 3
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**Table 3.** The linear regression analysis (with common intercepts) of N utilization efficiencies of dairy cows using data obtained between 1990 and 2002 (old dataset) and 2005-2019 (new dataset)

| | Equation [1] | | | $R^2$ | *P*-value | Eq. No |
|---|---|---|---|---|---|---|
| | Variable | Slope | Intercept | | | |
| Old dataset | Feces N = | $0.270_{(0.010)}$ N intake | $+ 12.0_{(8.5)}$ | 0.816 | 0.037 | 1a |
| New dataset | | $0.285_{(0.009)}$ N intake | | | | 1b |
| Old dataset | Urine N = | $0.407_{(0.018)}$ N intake | $+ 11.7_{(12.6)}$ | 0.832 | < 0.001 | 2a |
| New dataset | | $0.333_{(0.017)}$ N intake | | | | 2b |
| Old dataset | Manure N = | $0.673_{(0.015)}$ N intake | $+ 25.7_{(10.6)}$ | 0.905 | < 0.001 | 3a |
| New dataset | | $0.614_{(0.014)}$ N intake | | | | 3b |
| Old dataset | Milk N = | $0.102_{(0.0077)}$ N intake | $+ 61.0_{(12.6)}$ | 0.884 | < 0.001 | 4a |
| New dataset | | $0.128_{(0.0077)}$ N intake | | | | 4b |
| Old dataset | Retained N = | $0.221_{(0.0141)}$ N intake | $- 83.2_{(13.7)}$ | 0.517 | 0.009 | 5a |
| New dataset | | $0.250_{(0.0146)}$ N intake | | | | 5b |

[1]Values in subscript parentheses are SE.

**Table 4**
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**Table 4.** The linear regression analysis (with common slopes) of N utilization efficiencies of dairy cows using data obtained between 1990-2002 (old dataset) and 2005-2019 (new dataset)

| | Equation [1] | | | $R^2$ | *P*-value | Eq. No |
|---|---|---|---|---|---|---|
| | Variable | Slope | Intercept | | | |
| Old dataset | Feces N = | $0.275_{(0.009)}$ N intake | $+ 8.9_{(8.50)}$ | 0.816 | 0.035 | 6a |
| New dataset | | | $+ 18.3_{(9.10)}$ | | | 6b |
| Old dataset | Urine N = | $0.380_{(0.017)}$ N intake | $+ 22.9_{(12.7)}$ | 0.828 | <0.001 | 7a |
| New dataset | | | $- 9.5_{(13.9)}$ | | | 7b |
| Old dataset | Manure N = | $0.656_{(0.014)}$ N intake | $+ 31.9_{(10.9)}$ | 0.904 | <0.001 | 8a |
| New dataset | | | $+ 7.70_{(11.8)}$ | | | 8b |
| Old dataset | Milk N = | $0.012_{(0.0073)}$ N intake | $+ 57.7_{(12.7)}$ | 0.882 | 0.011 | 9a |
| New dataset | | | $+ 66.2_{(12.9)}$ | | | 9b |
| Old dataset | Retained N = | $0.233_{(0.0133)}$ N intake | $- 88.6_{(13.8)}$ | 0.516 | 0.035 | 10a |
| New dataset | | | $- 74.8_{(14.3)}$ | | | 10b |

[1]Values in subscript parentheses are SE.

**Table 5**
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**Table 5.** Relationships between experimental year and N utilization using the combined data (from 1990 to 2019, with 1990 defined as year 1 and 2019 as year 30)

| | Equation [1] | | | | |
|---|---|---|---|---|---|
| Variable | Slope | Intercept | $R^2$ | *P*-value | Eq. No |
| Feces N/N intake | $0.0010_{(0.0002)}$ EY | $+\ 0.294_{(0.0033)}$ | 0.116 | 0.131 | 11 |
| Urine N/N intake | $-\ 0.0043_{(0.0004)}$ EY | $+\ 0.449_{(0.0050)}$ | 0.419 | 0.001 | 12 |
| Manure N/N intake | $-\ 0.0032_{(0.0003)}$ EY | $+\ 0.743_{(0.0046)}$ | 0.451 | <0.001 | 13 |
| Milk N/N intake | $0.0021_{(0.0002)}$ EY | $+\ 0.213_{(0.0033)}$ | 0.238 | 0.025 | 14 |

[1]Values in subscript parentheses are SE; EY denotes experimental year.

**Table 6**


**Table 6.** Prediction of N output of dairy cows using total diet digestibility data (n = 476) collated from AFBI experiments undertaken from 2005 to 2019

| Equations[1] | $R^2$ | Eq. No |
|---|---|---|
| Fecal N output (g/d) = | | |
| $0.226_{(0.012)}$ NI + $47.0_{(12.8)}$ | 0.684 | 15a |
| $0.091_{(0.022)}$ LW + $2.64_{(0.23)}$ ECMY + $1.64_{(0.40)}$ DN + $1.2_{(17.3)}$ | 0.593 | 15b |
| Urine N output (g/d) = | | |
| $0.366_{(0.018)}$ NI – $10.1_{(17.9)}$ | 0.783 | 16a |
| $0.207_{(0.029)}$ LW + $1.15_{(0.34)}$ ECMY + $9.27_{(0.62)}$ DN – $212.3_{(34.2)}$ | 0.774 | 16b |
| Manure N output (g/d) = | | |
| $0.594_{(0.019)}$ NI + $36.7_{(12.4)}$ | 0.833 | 17a |
| $0.665_{(0.018)}$ NI | 0.833 | 17b |
| $0.277_{(0.040)}$ LW + $3.68_{(0.45)}$ ECMY + $11.32_{(0.81)}$ DN – $206.9_{(42.5)}$ | 0.779 | 17c |

[1]Values in subscript parentheses are SE. DN = diet N concentration, g/kg DM; ECMY = energy corrected milk yield, kg/d; LW = live weight, kg; NI = N intake, g/d

Table 7
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**Table 7.** Internal validation – evaluation of prediction accuracy for N output of dairy cows using one third of the present data and equations developed from two thirds of the present data (data collated from studies undertaken at AFBI from 2005 to 2019)

| Predictors[1] | N output, g/d | | | | | Predicted – actual N output, g/d | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Predicted | Actual | $R^2$ | RMSPE | SE | Mean | SD | Minimum | Maximum |
| Prediction of feces N output, g/d | | | | | | | | | |
| N intake | 161 | 159 | 0.48 | 2.05 | 17.4 | 2.44 | 23.3 | -67.1 | 65.2 |
| LW+ECMY+DN | 159 | 159 | 0.40 | 2.20 | 17.8 | 0.17 | 25.1 | -71.7 | 54.1 |
| Prediction of urine N output, g/d | | | | | | | | | |
| N intake | 175 | 179 | 0.49 | 3.87 | 28.1 | -4.76 | 43.9 | -124 | 93.6 |
| LW+ECMY+DN | 186 | 179 | 0.56 | 3.65 | 27.3 | 6.97 | 41.0 | -116 | 89.4 |
| Prediction of manure N output, g/d | | | | | | | | | |
| N intake | 337 | 338 | 0.72 | 3.61 | 34.0 | -1.52 | 41.1 | -120 | 96.7 |
| LW+ECMY+DN | 347 | 338 | 0.52 | 4.72 | 38.7 | 8.71 | 53.1 | -137 | 128 |

[1]DN = diet N concentration, g/kg DM, ECMY = energy corrected milk yield, kg/d, LW = live weight, kg, RMSPE = root mean square prediction error

Figure 1
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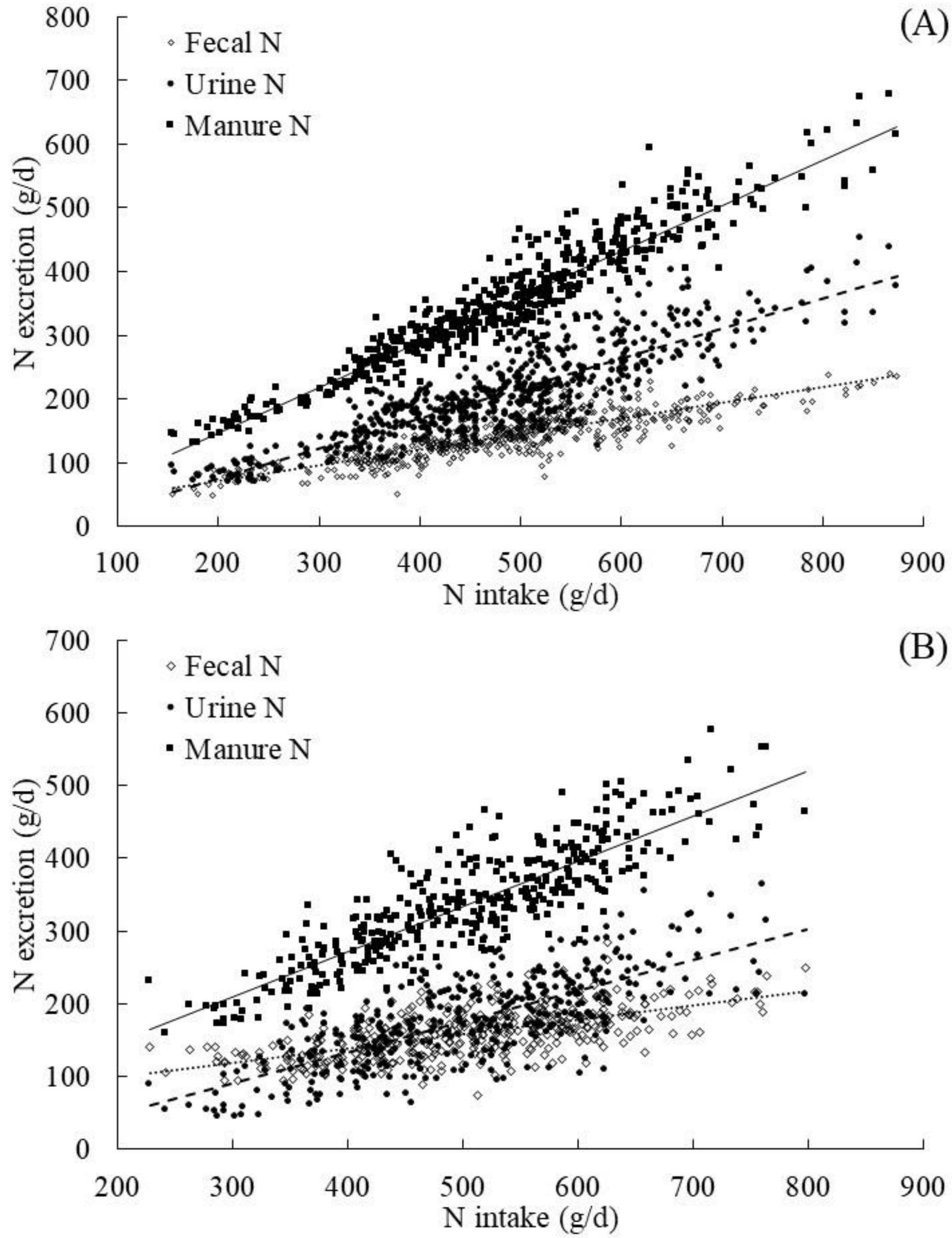


**Figure 1.** Relationships between N intake and N excretion using data of dairy cows collated from experiments undertaken at AFBI from 1990 to 2002 (old dataset, A) and from 2005 to 2019 (new dataset, B)

**Figure 2**
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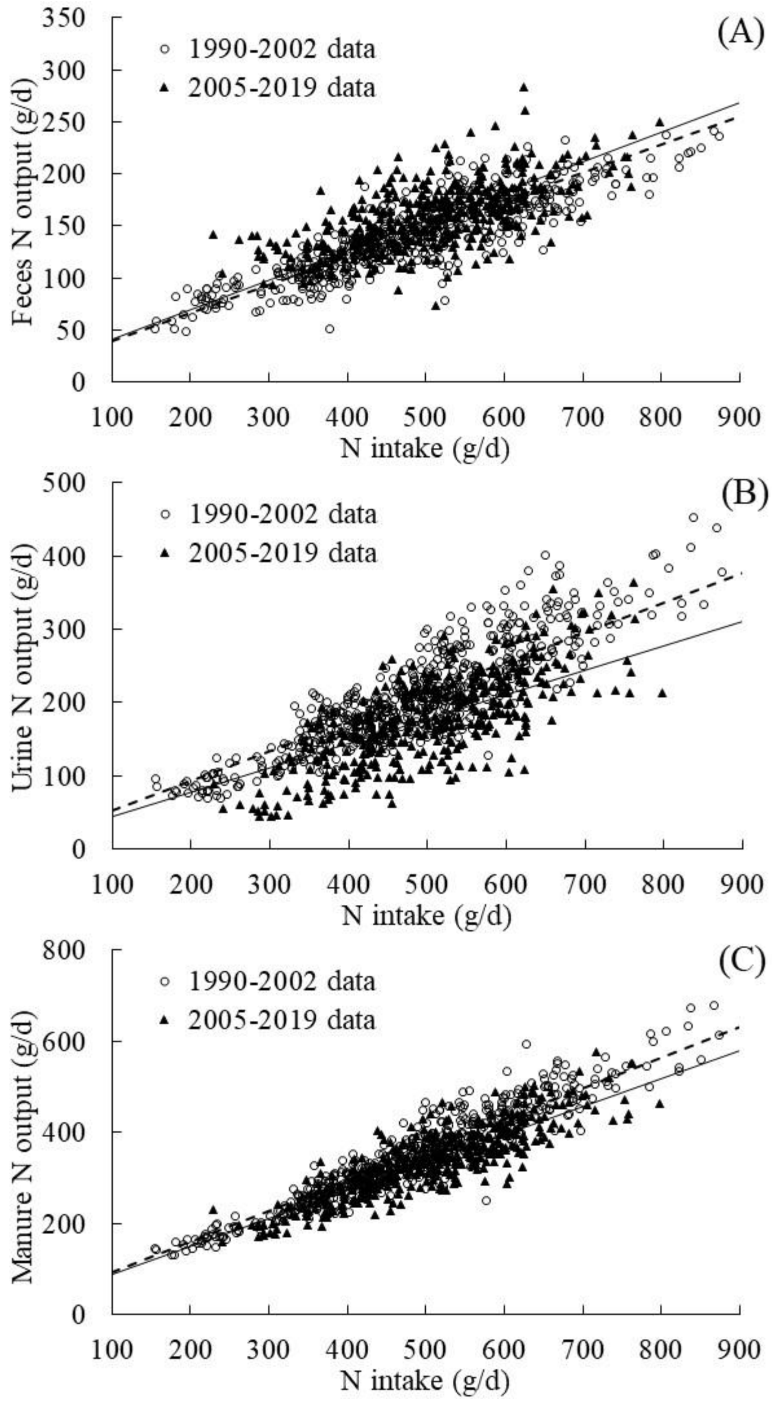


**Figure 2.** The comparison of N utilization efficiencies of dairy cows using data obtained between 1990-2002 (old dataset, dashed line) and 2005-2019 (new dataset, solid line) and the linear regression of N intake against N excretion in feces (A), urine (B) and total manure (C)

Figure 3
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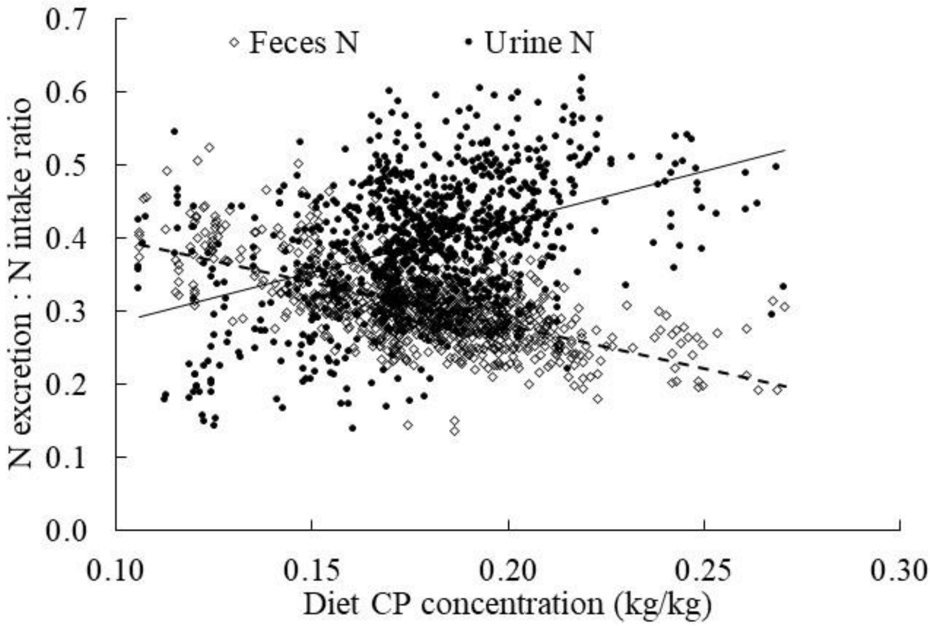


**Figure 3.** The relationships between diet crude protein (CP) concentration and N excretion ratios in feces (dashed line) and urine (solid line) using the combined data of old and new datasets in the present study

Figure 4
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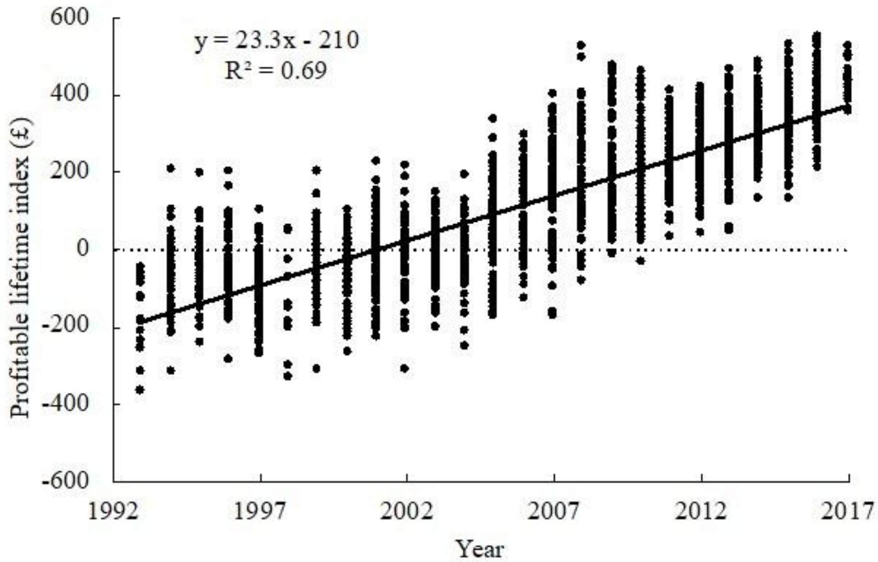


**Figure 4.** The improvement in the profitable lifetime index (base year - 2018) of Holstein dairy herd in the research farm of AFBI from 1993 to 2017