Dry matter intake, methane emissions and microbiome profiles as new traits for feed efficiency

***J. López-Paredes*[1], *A. Saborío-Montero*[2], *N. Charfeddine*[1], *J.A Jiménez-Montero*[1] *and O. González-Recio*[23]**

[1]*Spanish Holstein Association, CONAFE*
[2]*Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria*
[3]*Universidad Politécnica de Madrid*

Abstract

This study estimated the genetic parameters for feed efficiency, dry matter intake, methane production, and ruminal microbiome profile in order to incorporate feed efficiency into the breeding goal. Data consisted of 11,042 weekly averaged records for dry matter obtained from 551 cows in 5 farms, as well as 4,624 methane emission measurements using a non-dispersive infrared methane detector installed within the feed bin of the automatic milking system during 14 to 21 days period from 1,501 cows in 14 farms. In addition, ruminal content was extracted from 437 cows with methane phenotypes. Data for milk yield, protein yield, fat yield, fertility, body depth, rump width, chest width and predicted live weight from 11,228 cows were included in the analysis. Heritability and genetic correlations were estimated from bivariate models within a single step framework using AIREML. The heritability estimate for dry matter intake was moderate (0.16±0.03), with a genetic correlation with milk yield of 0.41 (0.11), and with type traits ranging from 0.25 to 0.49. Heritability estimates for methane production (0.17±0.05) and methane concentration (0.18±0.04) were moderate, and their genetic correlations with milk yield were close to zero (-0.05±0.11 and 0.04±0.11). Higher genetic correlations were estimated with type traits, ranging from 0.14 to 0.59. Genetic correlations between methane traits and dry matter intake were positive and ranged from 0.20±0.48 to 0.27±0.43. This suggests a correlated response in methane emissions when selecting for more ravenous animals. The microbiome aggregated variables showed higher positive genetic correlation with methane traits (0.53±0.19 and 0.87±0.19), and dry matter intake (0.32±0.36). These results highlight an important interrelationship between methane production and dry matter intake, with a significant role of the microbiome composition. The results suggest that selecting cows that make a more efficient utilization of energy intake is feasible through selection on correlated type traits such as body capacity, stature, and body depth, without compromising high productive performance. Selection for lower methane emissions is expected to have similar results. The inclusion of the microbiome composition may assist on a more efficient selection on both, lower emissions and higher feed efficiency. However, proper weights must be applied in the selection indices to maintain current genetic trends on productive and functional traits.

Key words: Methane, microbiome, feed efficiency, breeding, variance components.

Introduction

Genetic improvement of feed efficiency in cattle, which is cumulative and permanent, is a mean to achieve efficiency gains for a more profitable and sustainable production system (Berry and Crowley, 2013). The inclusion of traits related to feed efficiency into the breeding goal in dairy cattle and their improvement will impact on the herd profitability, where feed accounts for the largest part of operating costs in dairy production (>40%, Connor, 2015). In addition, the decrease of feed demand will rise the environmental sustainability of livestock farming through a minor land and resources intended for animal feeding. Moreover, it will involve a reduction of the environmental impact belong to dairy farming, which account the 13% of global greenhouse emissions (Leip et al., 2010). Ruminant are considered among the most important source of global CH_4 emissions from livestock, which is equivalent to producing around 4,623 Mt of CO_2e per year (Gerber et al., 2013) and which represent a loss between 2% and 12% of gross energy intake (Johnson and Johnson, 1995). On the other hand, CH_4 has an estimated lifetime of 10 years (IPCC, 2013) and hence, its mitigation would be possible in a short period of time. Several studies have analyzed traits such as dry matter intake or methane emissions and their inclusion into dairy cattle breeding program (Berry and Crowley, 2013; de Haas et al., 2016; González-Recio et al., 2020) reporting that their improvement thought genetic selection is feasible. However, there is still a lack of reported values of correlations with production and type traits in a large dataset such as Manzanilla-Pech et al. (2021). For instance, it is necessary to clarify the possible consequences of selecting for greater feed efficiency on other production and type traits.

Other proxies should be analyzed to achieve a greater genetic response. In recent years, efforts have been made to characterize the rumen microbiome and its functionality (Saborío-Montero et al., 2020), with the aim of implementing nutrition and selective breeding strategies to modulate it. The ruminal microbiota composition is partially controlled by the host genotype, and both affect important traits in livestock that are related to efficiency and sustainability, including methane production (Roehe et al., 2016; Gonzalez-Recio et al., 2018). Animal breeding can modulate the ruminal microbiota through selection and to achieve a more efficient microbial composition that reduces the use of natural resources and generates less methane emissions without impairing health and productivity (González-Recio et al., 2020; López-Paredes et al., 2020).

The aims of this work were to estimate the genetic parameters of dry matter intake, methane concentration and methane production, and microbiome composition variable and their genetic correlation with other traits as production, functional, and type traits which are related to the animal body size.

Materials and Methods

Data Description of Dry Matter Intake.

Data from 551 cows belonging to five farms located in Galicia, Asturias, and Catalonia, Spanish regions were collected from 2004 to 2020. The data consisted of 11,042 records weekly averaged. Records lower than 5 kg/d and higher than 60 kg/d were removed from the data.

Data Description of methane.

Data of CH_4 from 1,501 cows in 14 commercial farms from the Spanish regions of Basque Country, Cantabria, Girona and Navarre were collected from May 2018 to June 2019 with an automatic milk system (AMS). Methane was recorded daily during periods ranging from 14 to 21 d in each farm overlapping with an official test day record.

Methane emissions concentration (MeC) was measured using a non-dispersive infrared methane detector (The Guardian® NG infrared gas monitor; Edinburgh Instruments Ltd., Livingston, UK) installed within the feed bin of the AMS (Hammond et al., 2016). Methane concentration was calculated from the eructation peaks (Sorg et al., 2018; Rey et al., 2019), and were then averaged per cow and week and expressed in ppm (MeC). Methane production (MeP) expressed in g/d was calculated from MeC (expressed in volume ppm) based on the equation by Chagunda et al. (2009), where live weight (LW) were estimated from five traits following Pérez-Cabal and Charfeddine (2016). A detailed description may be found in López-Paredes et al. (2020).

Data description of microbiome composition

Principal component analysis (PCA) was used to reduce the dimensionality and to aggregate the metagenome variance into few variables. This was independently performed for the taxonomy and functional data sets. First, principal component scores of compositional description of ruminal microbiome (PC_1) described by Saborío-Montero et al. (2021) were used as phenotype explaining 31% of the variability at phylum level of ruminal microbiome composition. A comprehensive study of the sampling and the statistical analyses of the metagenome data was presented in Saborío-Montero et al. (2020)

Data Description of production, functional and type traits.

Milk yield (8,850) and type traits (8,470) from cows belonging to the 19 farms with methane or DMI phenotypes were included in the analysis to estimate the genetic correlations between those and efficiency traits. The traits included in the analysis were milk yield (MY), protein yield (PY), fat yield (FY), fertility expressed in days open (Fert), live weight (LW), body depth (BD), chest width (CHW), rump width (RW) at first lactation.

Genomic Data.

One thousand three hundred and thirty-two cows were genotyped using the Illumina EuroG10K chip (Illumina, San Diego, CA, USA). Besides, 314 males were genotyped using Illumina BovineSNP50 chip (Illumina, San Diego, CA, USA). Low density genotypes were imputed to 54,609 SNPs using BEAGLE software (Browning et al., 2018), as described in Jiménez-Montero et al. (2013) using the Spanish reference population provided by CONAFE. The SNPs that were not in Hardy-Weinberg equilibrium (P<0.05) had lower allele frequency than 0.05, or were located in the sex chromosome were filtered out. As a result, a total of 41,228 SNPs were considered for further analysis.

Variance Component Estimation

A bivariate Single-step genomic REML which combines the pedigree and genomic relationships was implemented to estimate MeC, MeP, PC_1, and DMI, and other secondary production and type traits variance components and correlations following the model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z_h}\mathbf{h} + \mathbf{Z_u}\mathbf{u} + \mathbf{Z_p}\mathbf{p} + \mathbf{e}$$

where **y** is a $n \times 1$ vector of phenotypic records for the trait of interest, **b** denotes the fixed effects for each model with incidence matrix X. For MeC, MeP, fixed effects were parity, days in milk expressed in moths, and month of calving. For PC_1 fixed effects were herd-year, days in milk expressed in months, month at calving and number of calving effect, For DMI, and production and type traits fixed effects were comparison group (Herd-year), days in milk expressed in months, month at calving and the effect of partity-age at calving, h is the vector of herd-week-robot effect included as random effect for MeC and MeP, with incidence matrix Z_h, u is the vector of direct animal genetic effects with association matrix Z_u, with u ~ $N(0,H\sigma_a^2)$ where H is a relationship matrix based in the pedigree and genomic relationship suggested by Misztal et al. (2009), Aguilar et al. (2010) and Legarra et al. (2014). Then, p is the vector of (within lactation) permanent effect for MeC, MeP and DMI, and e denotes the vector of residuals distributed as $N(0,I\sigma_e^2)$. All the analyses were performed using aireml.f90 from the BLUPF90 suit of programs (Misztal et al., 2002). Only significant effects were included in the genetic model (P<0.05) determined by the analyses of variance using lmerTest Package in R (Kuznetsova et al., 2016).

Results & Discussion

Descriptive statistics

Table 1 shows the number of cows, number of records, mean and standard deviation of methane traits, DMI and microbiota PC_1. The MeC mean was 1.288 ppm reporting higher values than 180.22 mg/kg reported by Pickering et al. (2015) using a laser methane detector. Negussie et al. (2017) estimated higher values of MeC of 3,919 ppm (SD 445) and MeP of 555 L/d (831 g/d) using photoacoustic spectroscopy. The MeP mean was 182.5 g/d. This value was lower than other recent studies. For instance, Manzanilla-Pech et al. (2021) estimated 392.3 g/d, Pszczola et al., (2017) estimated 279 g/d (sd 67.7). Manzanilla-Pech et al. (2021) reported a comparison of the different methodologies employed in four countries. The results oscillated from 345 g/d (Denmark) to 466 g/d (Australia) with correlation between GSMet (Methane standardized by countries) and MeP, of 0.99. This comparison of different methodologies has been studied in recent years in order to validate the results from different methods. Garnsworthy et al. (2019) showed correlations from 0.72 to 0.89 and concordances (Lin's concordance correlation) from 0.30 to 0.88 between the different methodologies yielding the largest variability between the various methods employed. Our differences with other countries may also be explained due to recording under commercial conditions. The placing of the tube of the device into the feed bin of the AMS may have an impact on the records of methane, since the cloud of the eructed gas may not be entirely registered (López-Paredes et al., 2020 and González-Recio et al., 2020). Thus, it is necessary to include a herd-robot effect in the statistical models to adjust for these differences. Furthermore, those lower values may be partly explained by the larger proportion of concentrate in Spanish ration compared to other European countries, as shown in FAO (2014).

Averaged values for DMI were 22.85 kg/d (SE=4.64). These values were higher than 14.80-20.17 kg/d estimated by Li et al. (2016) in 717 Holstein primiparous cows from Denmark, Finland, and Sweden, the 15.7 kg/d (SE=3.7) reported by Manzanilla Pech et al. (2014) or the 18.5 kg/d estimated by Veerkamp and Thompson (1999). Other authors reported similar values such as 21.3 kg/d Manzanilla-Pech et al. (2021) after merging data from seven different countries and production systems.

Table 1. Descriptive statistics for methane concentration (MeC, ppm), methane production (MeP, g/d), dry matter intake (DMI, kg/d), and microbiome trait (PC_1).

	no.cows	no.records	Mean (SD)
MeC	1,341	4,624	1.288(467.00)
MeP	1,500	4,624	182.5(67.00)
DMI	551	11,042	22.85(4.64)
PC_1	437	437	-0.067(5.028)

Table 2 shows the heritability, additive variance, permanent effect variance, and residual variance for MeC, MeP, PC_1, and DMI. Heritabilities of MeC were lower than 0.26 (0.11) estimated for Denmark population and similar to 0.15(0.15) for The Netherlands population provided by Difford et al. (2018) who estimated higher repeatability for MeC (0.47-0.80).

The estimated heritability (0.17-0.18) was low-moderate for methane traits involving a genetic component in individual methane emissions. It was slightly lower than the one previously reported by Manzanilla-Pech et al. (2016) of 0.23, and 0.25 estimated by Zetouni et al. (2018). Repeatability values suggest an important individual component for methane emissions when the measurements are taken within short periods of time. Sampling periods were from 2 to 3 weeks' periods, shorter than other studies such as Pszczola et al. (2017) or Breider et al. (2019) which reported higher h^2 using random regression models. Therefore, longer periods of sampling should be investigated further in future studies.

Microbiome variable (PC_1) showed the highest heritability (0.42) among the traits studied here. This result suggests that the core rumen microbiome is heritable as reported by Roehe et al. (2016), Difford et al. (2018), and Saborío-Montero et al. (2020).

Table 2. Additive variance (σ^2_a), permanent variance (σ^2_p), residual variance(σ^2_e), heritability (h^2) and repeatability (r^2) of methane concentration (MeC), methane production (MeP), first principal component (PC_1) of the microbiome composition, and dry matter intake (DMI).

		MeC	**MeP**	**PC_1**	**DMI**
σ^2_a	Mean	26,941.00	678.38	8.88	2.51
	s.e	10,527.00	232.25	4.07	0.53
σ^2_p	Mean	10,0460.00	2,025.00		3.95
	s.e	10,183.00	217.98		0.46
σ^2_e	Mean	53,696	1,080.80	11.71	8.78
	s.e	1,456.90	29.34	4.03	0.12
h^2	Mean	0.17	0.18	0.42	0.16
	s.e	0.05	0.04	0.21	0.03
r^2	Mean	0.70	0.71		0.42
	s.e	0.02	0.02		0.02

Variance of MeC is expressed in ppm^2, MeP is expressed in $(kg/d)^2$, and DMI is expressed in $(kg/d)^2$.

Heritability for DMI was 0.16 (0.03) which is lower than the values of 0.24(0.02) reported by Manzanilla-Pech et al. (2021) in a population of 2,360 cows belonging to farms from 6 different countries, and 0.19 and 0.24 estimated by Berry et al. (2014) from Canada and Australia in an international evaluation of feed intake. The reduced data set and the unconnected periods of recording could explain the obtained lower estimates. Large datasets are scarce in literature due to the complexity and cost associated to the feed intake records operations. To obtain a larger dataset the international collaboration should be considered in order to disentangle the genetic component of DMI and other traits related to feed efficiency. Repeatability of DMI was similar to the values reported by Manzanilla-Pech et al. (2021) in a larger dataset showing the existence of individual component for DMI. Repeatabilities were higher for methane traits compared to DMI, possibly caused by longer sampling periods for DMI and larger number of records.

Table 3 shows the genetic correlations between MeC, MeP PC_1 and DMI, with production, fertility and type traits related to capacity of the cow. Correlation between MeC and MeP was close to 1 suggesting that both traits are similar and highly correlated, leading to similar genetic responses regardless the trait used. Note that this correlation might be overestimated due to MeP was an indirect estimate of the actual methane production and it was calculated from MeC and estimated LW (López-Paredes et al., 2020). Methane traits were moderately correlated with PC_1 which clustered the animal with higher proportion of eukaryotes (Saborio-Montero et al. 2021).

Table 3. Genetic correlations (S.E) of milk yield (MY), fat yield (FY), protein yield (PY), fertility (Fert), live weight (LW), rump width (RW), chest width (CHW), Stature (STA), body depth (BD), and body condition score (BCS), with methane concentration (MeC), methane production (MeP), first principal component of the microbiome composition (PC_1), and dry matter intake (DMI).

	MeC	**MeP**	**PC_1**	**DMI**
MeC	-	0.97(0.03)	0.42(0.21)	0.20(0.48)
MeP	-	-	0.83(0.13)	0.27(0.43)
PC_1	-	-	-	0.32(0.36)
MY	-0.05(0.11)	0.04(0.11)	0.04(0.28)	0.41(0.11)
FY	0.30(0.14)	0.32(0.13)	0.18(0.72)	0.50(0.11)
PY	0.07(0.13)	0.13(0.12)	-0.02(0.31)	0.53(0.19)
Fert	0.02(0.25)	0.02(0.21)	0.04(0.62)	0.09(0.14)
LW	0.29(0.17)	0.62(0.17)	0.42(0.21)	0.41(0.13)
CHW	0.31(0.18)	0.58(0.16)	0.05(0.50)	0.49(0.16)
RW	0.18(0.12)	0.09(0.11)	0.42(0.21)	0.25(0.11)
STA	0.21(0.16)	0.45(0.20)	0.35(0.46)	0.34(0.12)
BD	0.14(0.13)	0.49(0.12)	0.35(0.61)	0.25(0.11)
BCS	0.25(0.16)	0.32(0.14)	-0.15(0.37)	0.22(0.19)

Correlations ranged from 0.42 to 0.83, consistent with the value of 0.74 reported by Saborio-Montero et al. (2021) between MeP and PC_1, which exposed that there is a relationship between the relative abundance of eukaryotic microorganisms and the methane production. This relationship and the high heritability of PC_1 would allow a high accurate selection of those animals which enhance the mitigation of the methane emissions.

Correlations between MY and methane traits were close to zero from -0.05 to 0.11, without a detriment on MY when selecting for lower emissions. These values were lower than the ones reported in the literature as 0.43(0.10) reported by Lassen and Løvendahl (2016) between MeP and fat-protein corrected milk using bivariate linear animal models with similar size of the data set. Breider et al. (2019) reported higher values ranged from 0.38 to 0.57 (with large standard errors (0.42-0.52), in a random regression bivariate model from 32 to 52 week. Breider et al. (2019) analyzed these correlations showing that in early lactation, the correlation between MY and CH_4 production may be negative because no CH_4 is produced from the body energy reserve mobilization. Later in lactation, energy for milk production originated from DMI, results in CH_4 production. This yields positive correlations between MY and MeP. Pszczola et al. (2019) estimated a low correlation of 0.15(SE=0.06) between MY and MeP in a random regression model. The correlations between FY and MeC and MeP shows a moderate correlation with FY following similar trends as Dijkstra et al. (2011) and van Lingen et al. (2014). This suggests a possible increase of emitted CH_4 per cow when breeding for larger FY. Correlations close to zero were found between CH_4 traits and PY. The correlations between CH_4 traits and Fert were lower than the ones reported in a previous study (López-Paredes et al., 2020) with larger SE of the estimates. Nonetheless, according to our results we don’t expect an impairing response on fertility if including CH_4 emissions into the breeding goal, following the exposed by Gonzalez-Recio et al. (2020) which points toward a favourable relationship between fertility traits and methane emissions. Correlations between type traits and CH_4 traits were low to moderate, it suggests that selecting for greater size animals, with larger rumen capacity, could consequently lead to larger values for MeP. These results confirm the ones previously reported by López-Paredes et al. (2020) using EBV´s correlations from Calo´s approximation (Calo et al., 1973). These values were higher than the ones estimated by Zetouni et al. (2018) who reported correlations with MeP of -0.20(0.13) for CHW, 0.01(0.08) for STA, -0.03(0.12) for BD, with larger and negative correlation for BCS, -0.28(0.10) with an opposite trend to our estimates. Zetouni et al. (2018) showed that BCS contributes to better fertility status in dairy cattle. Therefore, it seems reasonable to consider that cows with fewer reproductive issues would also produce less CH_4. However, less feed efficient cows make less efficient utilization of the feed with lower production but better BCS and fertility, and higher fat-tissue deposition, which explains the positive correlation with BCS (López-Paredes et al., 2020). Further studies with larger data set should be developed to confirm this relationship.

Microbiome variable (PC_1) presented moderately high correlation with methane traits, and close to zero with DMI (with large SE) with greater correlations for type traits as LW, RW, STA, and BD and low correlations with production traits. PC_1 seems to be a variable capable of improving the reduction of CH_4 without a detriment of productivity of the animal. This leads to the use of PC_1 as an indicator of more efficient cows, since the loss of energy from CH_4 belonging to enteric fermentation is proportionally reduced. Saborio-Montero et al. (2020) defined PC_1 as a representative of the core rumen metagenome, to modulate the rumen metagenome towards greater efficiency and sustainability, although reproducibility across data sets needs to be confirmed.

Dry matter intake showed moderately high correlations with production traits expecting higher feed intake when selecting for more productive cows (higher MY, FY, and PY).

Correlation between DMI and MY was 0.41(0.11) similar to 0.44(0.13) reported by Manzanilla-Pech et al. (2016) in The Netherlands and United States joint population, and 0.48(0.05) by (Manzanilla-Pech et al., 2021) in a collaboration action between six countries (n=2,320). Veerkamp and Brotherstone (1997) estimated correlations of 0.34-0.59,0.59-0.55, and 0.86-0.80 between DMI and MY, FY, and PY, for heifers and heifers + multiparous cows, respectively. According to our results, non-favourable response might be obtained when selecting for lower DMI. for this reason, DMI, and production traits such as MY, FY, and PY, should be included in an appropriate selection index accounting for an appropriate economic weight with the aim of achieving a balanced genetic response of these traits. We found a correlation close to zero between Fert and DMI, hence a detriment of Fert is not expected with lesser DMI. On the other hand, DMI showed positive correlations with type traits expecting greater size, with correlations between DMI and LW and STA of 0.41, and 0.34, respectively, and capacity, with correlations of 0.49, 0.25, and 0.25 for CHW, RW, and BD, respectively. These results were in agreement with others values founded in the bibliography as 0.27 estimated between LW and DMI by Veerkamp and Brotherstone (1997), and 0.52(0.35) estimated by Vallimont et al. (2010). Manzanilla-Pech et al. (2016) estimated correlations of 0.33-0.57, 0.45-0.61, 0.26-0.49, 0.04-0.13, and 0.24-0.46 between DMI, and STA, CHW, BD, RW, and BCS respectively, for The Netherlands, and United States populations, respectively. According to these results, including type capacity traits into a selection index for feed efficiency is fully justified.

Conclusions

According to our results, selecting for more efficient cows is feasible using the proposed traits (methane, DMI, and microbiome variable) as proxies. Methane traits showed moderate h^2 and they were lowly correlated with milk yield. Hence, it is possible to select for a decrease of methane emissions without a detriment of milk yield. Methane traits showed moderate correlations with type traits related to capacity of the cow. Dry matter intake showed moderate heritability and large correlations with live weight, type traits related to capacity and production traits. The inclusion of microbiome variable may assist on a more efficient selection on both lower emissions and higher feed efficiency. Including those traits into a selection index should be a priority in the following years for breeding programs with an emphasis on data recording of phenotypes related to feed efficiency such as DMI and methane traits.

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