Animal breeding sustainability: the Italian Holstein experience

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Abstract

Livestock farming, especially dairy breeding, has a significant influence on environmental balance, accounting for about 50% of greenhouse gases (GHG) emissions from the primary sector. To increase the environmental sustainability of the dairy sector, even in the face of growing interests of the consumers towards this topic, a holistic approach is needed. Methane and carbon dioxide emissions have been shown to be heritable in cattle, providing the basis to apply genetic selection for their reduction. Furthermore, it is necessary to consider that GHG recording is complex, expensive and time consuming. In this context national breeding programs can provide relevant contributions. For this reason, the Italian Association of Holstein, Brown and Jersey breeders (ANAFIBJ) is working on data collection of innovative phenotypes and, in the future, to set-up routine recording in commercial dairy farms. Since 2018 ANAFIBJ, has started to record GHG data on young genotyped Italian Holstein bulls passing into the Genetic Center. For this purpose, the GreenFeed system (C-Lock Inc., Rapid City, SD) has been installed and used. In three years, a dataset of more than 11,200 phenotypic records collected on more than 200 young bulls has been set-up. Preliminary analyses showed that animals emit 223,6 g of CH4/d with a heritability (h2) of 0.396. Thanks to this experience ANAFIBJ has the intention to contribute further and set up a routine recording system for these phenotypes implementing experimental protocols to apply in commercial farms. For this purpose, Laser Methane Detector Mini (LMD, Crowcon, Abingdon, UK) is currently being tested at ANAFIBJ Genetic Center and a data collection protocol is under investigation. Once a standard protocol will be defined, individual CH4 emissions will be collected in 3,000 genotyped Italian Holstein dairy cows (some of them daughters of the young bulls recorded at the Genetic Center) distributed in 100 commercial farms throughout the country. At the Genetic Center, in addition, several phenotypes will be collected in order to better define the GHG data emission. Main biometric measures will be recorded and samples of ruminal fluid and faeces will be collected. Biological samples will be frozen and stored at -80°C, until instrumental and bioinformatic analysis. Activities in commercial farms and in experimental stations will allow to study the interaction between host and environmental microbiome, and to evaluate the reliability of faeces as a proxy of rumen sample. Furthermore, it will be possible to estimate the genetic parameters and to develop models for genetic and genomic evaluations of methane emissions.

Keywords: Greenhouse gas emissions, data-collection, Holstein, data-analysis, GreenFeed, laser methane detector.

Introduction

Agriculture and livestock sectors are recognized as important contributors to global temperature increase (Cassandro *et al*., 2013). Livestock farming, with particular regard to ruminants, is linked to GHG emissions due to enteric fermentation. Furthermore, livestock sector indirectly contributes to GHG emissions through activities related to feed production, manure spreading and storage, nitrogenous fertilisers, fossil fuels consumption and deforestation.

Methane and carbon dioxide emissions have been shown to be heritable, providing the basis to apply genetic selection for their reduction (Pickering *et al*., 2015; Lassen and Løvendahl,, 2016). Such program could be applied directly, by selecting for breath measurements, but also using indirect selection including indicator traits such as feed intake (de Haas *et al*., 2017). National breeding program can provide relevant contribution to reduce GHG emissions. The objective of this study was to present the ANAFIBJ experience and future perspective for GHG data collection and set up of a routine protocol for commercial and experimental Italian Holstein dairy farms. Furthermore, it will allow to study in deep the knowledge of the microbiome-host and environment-microbiome interactions and to evaluate the reliability of faeces as a proxy of rumen sample.

Material and methods

Animals involved in this preliminary study were young genotyped Italian Holstein bulls undergoing progeny test in the Genetic Center of the ANAFIBJ as reported by Callegaro *et al*. (2022). These young bulls had a genomic index included in the best 2% of the Italian Holstein population; they will be the future reproducers of the Italian Holstein breed. The ANAFIBJ Genetic Center is currently equipped with two instruments for individual impact recording: 1) GreenFeed *(C□Lock Inc., Rapid City, SD, USA*), and 2) The Laser Methane Detector (*LMD - Crowcon, Abingdon, UK*).

1. **The GreenFeed**, considered as an "Automated Head-Chamber System (*AHCS),* is an automated feeding station designed to measure daily CH_4 and CO_2 emissions (g/d) from ruminant's breath (Hristov *et al*., 2015). No more than 20 animals were housed in the GreenFeed box in order to ensure animal welfare and to avoid multiple animals at once. Each animal could visit the AHCS every six hours. At each visit the feed was unloaded a maximum of six times, for a total of 24 daily visits per animal. This set of traits included number of visits (NVG), carbon-dioxide daily emission (CO_2), methane daily emission (CH_4), average airflow (AIR) and average time (ATG).
2. **Laser Methane Detector Mini** (LMD). The LMD is a highly responsive, hand-held device that is pointed at an animal's nostrils and, based on infrared absorption spectroscopy, measures methane column density along the length of the laser beam (ppm*m) (Garnsworthy *et al*., 2019). This instrument is connected to a smartphone or tablet for data storing. During LMD data collection all ventilation or cooling systems inside the barn were turned off. LMD default settings were maintained with a measurement interval of 0.5 seconds (two values of CH_4 per seconds were measured). The operator was located in front of the standing

animal at a distance of 1.5 meters. Each measurement lasted 330 seconds. Each animal was recorded 3 times per day for 10 consecutive days for a total of 28 measurements per animal. The data generated make a list of CH_4 values accompanied by a unique date and time stamp and a value for the quality of the reflection of the laser beam. A single measurement consisting in a time series of CH_4 values of a single animal is called "profile". From each profile the raw mean (MEAN) of CH_4 and the mean of all peaks (P_MEAN) was calculated (Niero *et al.*, 2020).

GreenFeed data were relative to 221 Italian Holstein young bulls between 171 and 541 days of age. Data have been recorded in the period between May 2018 and April 2022 and each trial lasts on average 15 days.

LMD Genetic Center phenotypic data available belong to 18 Italian Holstein young bulls between 171 and 541 days of age for a total of 483 profiles. Records with intensity less than 100 were discarded. Profiles analysis was carried as reported by Sorg *et al.*, 2018. Data have been recorded in the period between January 2022 and June 2022.

Animals' biometric measures were also recorded. This set of traits included measures of body growth taken using electronic scales and stadiometers operated by qualified personnel as body weight (WEI), Body Condition Score (BCS), hearth girth (HG) and height (HT).

All young bulls were genotyped using various SNP chips resulting in 69,127 SNP. Genomic data were subsequently edited using the preGSf90 software (Aguilar *et al.*, 2010), removing SNP with call rate below 0.9 and minor allele frequency below 0.05. After editing, 61,591 SNP were available.

Results

Descriptive statistics and heritability estimates for the studied traits are reported in Table 1 and Table 2. Growth traits showed the largest heritability estimates (h^2), all being above or close to 0.40. While these traits are expected to be highly heritable, the estimates appeared larger compared to those found in literature. This could be due to the relatively small sample size. Heritability estimates for the emission traits were moderate to high, ranging from 0.241 for ATG to 0.480 for CO_2.

Discussion

Heritability estimates showed substantial genetic variation for the studied traits. The CO_2 and CH_4 emissions calculated on daily basis showed high heritability with the possibility of selection and therefore possible reduction of GHG emissions. The estimated values for the heritability of CH_4 and CO_2 were higher than those found in literature (Lassen and Løvendahl, 2016; Brieder *et al.*, 2019), although this could be due to the involvement of growing bulls rather than lactating cows and the limited sample size in the current study.

Table 1. Descriptive statistics (posterior means with posterior standard deviation) and heritability estimates for the traits analysed.

Trait[1]	Metric	N	Mean	SD	h^2
WEI	kg	885	309.3	77.48	0.445 (0.236)
BCS	score	849	3.0	0.33	0.512 (0.201)
HG	cm	715	157.3	14.15	0.441 (0.247)
HEI	cm	714	125.5	7.71	0.393 (0.225)
NVG	count	2817	3.9	1.71	0.360 (0.113)
CO_2	g/d	2817	6198.2	1103.88	0.480 (0.206)
CH_4	g/d	2817	223.6	51.83	0.396 (0.169)
AIR	L/s	2817	29.2	4.02	0.448 (0.088)
ATG	s	2817	329.3	87.49	0.241 (0.105)

[1]WEI: body weight; BCS: body condition score; HG: heart girth; HEI: height; NVG: number of visits at the GreenFeed; CO_2 daily carbon dioxide emissions; CH_4: daily methane emissions; AIR: average airflow at the visit; ATG: average time at the GreenFeed.

Table 2. Descriptive statistics for the traits analysed using Laser Methane Detector.

Trait[1]	Metric	N	Mean	SD
P_MEAN	ppm*m	18	53.9	30.62
MEAN	ppm*m	18	30.19	20.65

[1] P_MEAN: arithmetic mean of all peaks; MEAN: arithmetic mean of all values.

Conclusion

These preliminary results suggest that selection indexes could be implemented in order to reduce GHG emissions. ANAFIBJ is testing several experimental protocols in the Genetic Center; it is turning in a "Living Lab". Thanks to this experience ANAFIBJ wants to contribute further and set up a routine recording system implementing experimental protocols to be applied both in experimental and commercial farms to collect a critical mass of data useful for Italian Holstein selection. Individual CH_4 emissions will be collected in 3,000 genotyped Italian Holstein dairy cows distributed homogeneously in 100 commercial farms throughout the country. Some of these cows are daughters of the young bulls recorded at the Genetic Center. In addition, other phenotypes will be collected in order to better define GHG data emissions.

Furthermore, samples of ruminal fluid, faeces and buccal swabs will be collected. Rumen fluid will be collected using the Flora Rumen Scoop (Geishauser *et al*. 2012), a ruminal probe specially designed for cattle. The first 0.5 liter of the rumen fluid will be discarded to avoid saliva contamination, and the next 0.5 liter will be retained for sampling. After each sampling the probe will be washed with water, disinfected, rinsed and dried. This procedure will allow to avoid cross-contamination of rumen fluid between animals and to avoid interference of chemical products used for disinfection. Ruminal fluid samples will be stored in individual bottles at -80°C. At the same time buccal swabs and faeces will be sampled. Buccal swabs will be collected using salivary swabs. Faeces will be obtained by rectal grab sampling using disposable gloves and will be stored in plastic bags. Ruminal fluid, buccal swabs and faeces will be submitted for metagenomic analysis. Relative abundance of OTU (Operational Taxonomic Units) will be evaluated. These activities will allow to study in deep the knowledge of the microbiome-host and environment-microbiome interaction and to evaluate the reliability of faeces as a proxy of rumen sample.

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