Characterization and mitigation option of greenhouse gas emissions from lactating Holstein dairy cows in East China

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Abstract

**Background:** This study investigated greenhouse gas (GHG) emission characteristics of lactating Holstein dairy cows in East China and provided a basis for formulating GHG emission reduction measures. GreenFeed system was used to measure the amount of methane (CH\textsubscript{4}) and carbon dioxide (CO\textsubscript{2}) emitted by the cows through respiration. Data from a commercial cow farm were used to observe the effects of parity, body weight, milk yield, and milk component yield on CH\textsubscript{4} and CO\textsubscript{2} emissions.

**Results:** Mean herd responses throughout the study were as follows: 111 cows completed all experimental processes, while 42 cows were rejected because they were sick or had not visited the GreenFeed system 20 times. On average, lactating days of cows was 138 ± 19.04 d, metabolic weight was 136.5 ± 9.5 kg, parity was 2.8 ± 1.0, dry matter intake (DMI) was 23.1 ± 2.6 kg/d, and milk yield was 38.1 ± 6.9 kg/d. The GreenFeed system revealed that CH\textsubscript{4} production (expressed in CO\textsubscript{2} equivalent, CO\textsubscript{2}-eq) was found to be 8304 g/d, CH\textsubscript{4}(CO\textsubscript{2}-eq)/DMI was 359 g/kg, CH\textsubscript{4}(CO\textsubscript{2}-eq)/energy-corrected milk (ECM) was 229.5 g/kg, total CO\textsubscript{2} production (CH\textsubscript{4} production plus CO\textsubscript{2} production) was 19,201 g/d, total CO\textsubscript{2}/DMI was 831 g/kg, and total CO\textsubscript{2}/ECM was 531 g/kg. The parity and metabolic weight of cows had no significant effect on total CO\textsubscript{2} emissions (\(P > 0.05\)). Cows with high milk yield, milk fat yield, milk protein yield, and total milk solids yield produced more total CO\textsubscript{2} (\(P < 0.05\)), but their total CO\textsubscript{2} production per kg of ECM was low (\(P < 0.05\)). The total CO\textsubscript{2}/ECM of the medium and high milk yield groups was 17\% and 27\% lower than that of the low milk yield group, respectively.

**Conclusions:** The parity and body condition had no effect on total CO\textsubscript{2} emissions, while the total CO\textsubscript{2}/ECM was negatively correlated with milk yield, milk fat yield, milk protein yield, and total milk solids yield in lactating Holstein dairy cows. Measurement of total CO\textsubscript{2} emissions of dairy cows in the Chinese production system will help establish regional or national GHG inventories and develop mitigation approaches to dairy production regimes.

**Keywords:** Enteric methane emissions, GreenFeed system, Holstein dairy cows, Mitigation option, Production performance

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Background
Climate change caused by greenhouse gas (GHG) is a huge environmental challenge to mankind [1]. Manufacturing, agriculture, and electricity sectors are the primary sources of GHG emissions, with agricultural emissions accounting for about 24% of total emissions [2]. Livestock is a prime anthropogenic source of methane (CH4) emissions from the agricultural sector, accounting for 18% of global GHG emissions [3], of which ruminant livestock is responsible for 93% of all livestock GHG emissions globally [4], and the dairy cows have the largest GHG emissions [5]. Carbon dioxide (CO2) and CH4 are the first two components of GHG, and CH4 has 25 times more warming power in the near term than CO2 [2]. To indicate their global-warming potential in the atmosphere, CH4 emissions are commonly quantified in CO2-eq units [6]. Furthermore, the residence time of CH4 in the atmosphere is 12.2 years, which is much lower than that of CO2 [7]. CH4 reduction is the fastest way to quickly mitigate climate change in the short term; therefore, attention should be paid to the ruminant industry, especially the dairy industry.

Meeting the demand for animal protein products has become a primary challenge for global food security as the world’s population continues to expand [8]. Ruminants are almost the sole source of milk for humans, providing 644 million tons per year of fat- and protein-corrected milk, of which dairy cows contribute to 80% [9]. However, the anaerobic fermentation of fiber feed in the rumen inevitably produces CH4 and affects the climate. In addition, the production of CH4 will cause a loss of 5–14% of the total energy intake of dairy cows [9]. Milk output is estimated to double by 2050 as the global population continues to grow [10]. As the consumer demand for dairy products is increasing, the expansion of the dairy industry aggravates the accumulation of GHG in the atmosphere contributing to global warming [11]. Therefore, there will be an urgent need to avoid the negative effects on the environment and save dietary energy by reducing CH4 emissions from dairy cows [12]. This necessitates the improvement in the efficiency of dairy cow production resource utilization reducing the GHG emissions to ensure sustainable and clean dairy cow production in the future. Despite the advances in research on GHG emissions from animal husbandry in Europe and the United States, there is a dearth of a GHG emission database in China. Understanding these emissions requires a significant amount of basic research to establish a database. China lacks a local dairy cow GHG emissions database, and before this experiment, neither did China have the latest animal CH4 emission detection equipment (GreenFeed system). Furthermore, China did not measure the gas emissions from a large herd of cows. The purpose of this study was to accurately determine the GHG emissions of lactating Holstein dairy cows under normal feeding conditions using the GreenFeed system and to calculate the relationship between GHG emissions and parity, body weight, milk yield, and milk component yield. This would lay the foundation for determining the CH4 and CO2 emissions of lactating Holstein dairy cows in East China, as well as facilitating further studies on the GHG emission characteristics of lactating Holstein dairy cows and locally applicable GHG emission reduction approaches in China.

Materials and methods
Animals, diets, and experimental design
China’s dairy cows are mainly concentrated in the north, and milk production in the North China Plain accounts for 25% of the total milk production in China [15]. Hence, the test data from farms in the North China Plain would be more representative. The experiment was conducted at the Yinxiangweiye International Third Farm, which is a part of the Yinxiangweiye Group Co., Ltd. within Cao County, Shandong Province (34°83 N, 115°54E).

The 153 healthy lactating Holstein dairy cows were selected from this farm as experimental cows, and these animals were housed in a barn. The parities of 153 lactating Holstein dairy cows ranged from 2 to 5, the days in milk from 104 to 182 d, and milk yield from 25.9 to 53.7 kg/d.

The cows were kept in a freestall barn (200 m x 10 m) and had free access to drinking water and saltlicks. The cows were fed a basal TMR with a forage:concentrate ratio of 40:60 on a dry matter (DM) basis, and the composition of the TMR was the same throughout the experiment (Table 1). The TMRs were provided three times daily in 4:3:3 proportions by an automatic feed wagon to guarantee ad libitum intake (aiming at 10% refusals), with feeding times of approximately 08:30, 15:30, and 23:30 h.

The whole experiment was completed in 120 d with measurements of enteric CH4 and CO2 emissions, DMI, milk production, milk composition, and body weight of cows. There were four experimental periods, each 30 d
long, and in experimental periods 1–4, 40, 40, 40, and 33 cows were randomly selected for measurement, respectively. The data of lactating Holstein dairy cows was divided into three groups according to parity, metabolic weight (MW), milk yield, milk fat yield, milk protein yield, and total milk solids yield. In addition, to be divided into three groups according to second parity (SP), third parity (TP), and fourth and above parity (FAP), the others were divided into three groups based on the standard deviation (SD): less than mean – 0.5 × SD, mean ± 0.5 × SD, and more than mean + 0.5 × SD. According to MW, milk yield, milk fat yield, milk protein yield, and total milk solids yield, the cows were divided into low metabolic weight (LMW), medium metabolic weight (MMW), and high metabolic weight (HMW) groups; low milk protein yield (LMPY), medium milk protein yield (MMPY), and high milk protein yield (HMPY) groups; low total milk solids yield (LTMSY), medium total milk solids yield (MTMSY), and high total milk solids yield (HTMSY) groups, respectively.

**Measurement of methane and carbon dioxide emissions from lactating Holstein dairy cows using GreenFeed system**

The GreenFeed system is the latest technique to directly measure the enteric greenhouse gas emissions and other gases (H₂, O₂) from animals. It is non-invasive, has a short measurement time and can be used in a large group of animals [7]. In the present study, it was necessary to ensure that the 153 cows were in a natural feeding state to obtain the actual gas emissions data of Chinese lactating Holstein dairy cows. Therefore, the GreenFeed system was the most suitable for the experiment.

**Determination of methane and carbon dioxide emissions from lactating Holstein dairy cows**

Respiratory gas exchange measurements were performed over the entire experimental period. Two GreenFeed units (C-Lock Inc., Rapid City, SD, USA) were permanently available for measuring gas emissions from cows according to the methods of Huhtanen et al. [16]. Before the measurements, the cows were allowed to adapt to the units for 5 d. Span gas (O₂, CO₂, and CH₄) and zero gas (N₂) calibrations were performed once a week. The standard gases consisted of two concentrations of O₂ (2000 and 2100 ppm), 1500 ppm each of CO₂ and CH₄ for span gas, and 100% N₂ (99.999% pure) for zero gas. AC O₂ recovery test was conducted every 2 weeks during the entire experiment; the mean recovery was 100 ± 5%. Airflow was maintained above the manufacturer’s recommended rate of 26 L/s by cleaning the air filter when the flow rate approached this level. Alfalfa pellets (Ningxia Guyuan Forage Co., Ltd., Guyuan City, NX, CHN) were offered as bait feed to regularly entice the cows to visit the GreenFeed system. The weight of the alfalfa pellets obtained when each cow visited the units was recorded and used to calculate the DMI. The units were configured to allow each animal to visit at a minimum of 5-h intervals. During each visit, the cows were given eight drops of 30 g alfalfa pellets every 40 s, and the head position remained relatively stable for more than 3 min as a valid visit. More than 20 valid data points were ensured for each cow, and the average value was calculated as the final data; otherwise, it would be eliminated.

| Ingredient and chemical composition of the basal diet fed to lactating Holstein dairy cows |
|-----------------------------------------------|
| **Items** | **Content, % of DM** |
| Ingredient composition a | |
| Corn silage | 21.10 |
| Alfalfa hay | 14.75 |
| Oatgrass hay | 3.46 |
| Dandelion hay | 0.69 |
| Steam-flaked corn | 14.77 |
| Soybean meal | 14.25 |
| Corn flour | 11.80 |
| Beet pulp | 5.11 |
| Whole cottonseed | 4.39 |
| Rapeseed meal | 2.56 |
| Extruded soybean | 1.64 |
| Mineral-vitamin premix b | 5.48 |
| Calculated chemical composition | |
| OM | 97.80 |
| CP | 16.78 |
| EE | 5.48 |
| NDF | 32.75 |
| ADF | 22.09 |
| Ca | 0.82 |
| P | 0.46 |
| NEL, Mj/kg | 7.46 |

| a | OM dry matter, OM organic matter, CP crude protein, EE ether extract, NDF neutral detergent fiber, ADF acid detergent fiber, Ca calcium, P phosphorus, NEL net energy values were estimated based on NRC (2001) |
| b | The premix contained 140 g/kg of Mg, 122 g/kg of Ca, 93 g/kg of Na, 50 g/kg of K, 48 g/kg of Fe, 24 g/kg of P, 2 g/kg of S, 999 mg/kg of Zn, 580 mg/kg of Mn, 360 mg/kg of Cu, 180,070 IU of VA, 30,000 IU of VD and 601 IU of VE |
Data and sample collection

Collection and analysis of the feed samples

During the entire experiment, the feed offered and refused were recorded daily for the barn to calculate the average feed intake of the cows. The TMR samples were collected once a week and were combined to obtain representative samples for the entire period of the experiment for analysis. The methods used were DM (Method 942.05; AOAC International, 1995) [17], CP (Method 990.03; AOAC International, 2000) [18], amylase-treated NDF (Van Soest et al., 1991) [19], EE (Method 2003.05; AOAC International, 2006) [20], ADF (Method 973.18; AOAC International, 2000) [18], ash (Method 942.05; AOAC International, 2000) [18], and minerals (Method 985.01; AOAC International, 2000) [18]. The GE content was determined using automatic oxygen bomb calorimetry (Parr Instrument Inc., Moline City, IL, USA). The ingredian and chemical composition of the basal diet were shown in Table 1.

Determination of body weight, milk yield, and milk composition of lactating Holstein dairy cows

Cows were weighed before morning feeding with an electronic loadometer (Zhengfeng Loadometer Co., Ltd., Shanghai, CHN) on the second day after completing the measurement of gas emissions. Cows were milked three times daily at 08:00, 15:00, and 23:00 h; milk yield was digitally logged with gravimetric milk recorders (Afimilk Co., Ltd., Kibbutz, IL) at each milking. Milk samples were collected from three consecutive milkings on the second day after completing the measurement of gas emissions, and the collected milk samples were mixed in a ratio of 4:3:3. The samples (~ 50 mL) were preserved with 6% potassium dichromate (K₂Cr₂O₇), stored at 4 °C, and analyzed within 3 d. Finally, the milk samples were submitted to the Shandong Province Testing Center for the analysis of SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). The metabolic weight, milk yield, FCM yield, ECM yield, milk component (fat, protein, and total milk solids). Fat-corrected milk (FCM 4%, kg/d) = 0.4 × milk yield (kg/d) + 15 × fat yield (kg/d) [21]. The energy-corrected milk (ECM, kg/d) = milk yield (kg/d) × [(38.3 × %fat × 10 + 24.2 × %protein × 10 + 16.54 × %lactose × 10 + 20.7) ÷ 3140] [22].

Calculations

The conversion factor [2] was used to convert CH₄ to CO₂ equivalents. The total CO₂ emissions were equal to the combined CH₄ and CO₂ exhaled by lactating Holstein dairy cows.

\[ \text{CH}_4\text{CO}_2(g/d) = \text{CH}_4\text{emissions(g/d)} \times 25 \quad (1) \]

TotalCO₂(g/d) = CH₄(CO₂-eq)(g/d) + CO₂emissions(g/d) \quad (2)

Statistical analysis

A total of 153 lactating Holstein dairy cows were continuously adjusted according to management standards of this experimental farm based on hoof disease, mastitis, and other reasons. The 42 cows that made less than 20 valid visits to the system were eliminated, and 111 cows completed all data collection. All data were screened for normality using the UNIVARIATE procedure of SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). The metabolic weight, milk yield, FCM yield, ECM yield, milk component (fat, protein, and total milk solids) were shown in Table 1.

Table 2 Feed intake, milk production and composition and carbon dioxide emission of lactating Holstein dairy cows (n= 111)

| Items | Mean | Minimum | Maximum | SD |
|-------|------|---------|---------|----|
| Age, months | 51.7 | 36.3 | 89.9 | 12.7 |
| Parity number | 2.8 | 2.0 | 5.0 | 1.0 |
| Days in milk, d | 138 | 104 | 182 | 19 |
| Metabolic weight, kg | 136.5 | 116.4 | 160.1 | 9.5 |
| Dry matter intake, kg/d | 23.1 | 17.6 | 33.7 | 2.6 |
| Milk yield, kg/d | 38.1 | 25.9 | 53.7 | 6.9 |
| Feed efficiency, kg/kg | 1.65 | 0.62 | 2.32 | 0.29 |
| Milk fat yield, g/d | 1414 | 545 | 2222 | 272 |
| Milk protein yield, g/d | 1247 | 446 | 1991 | 236 |
| Total milk solids yield, g/d | 4720 | 1811 | 6295 | 786 |
| FCM yield, kg/d | 36.4 | 29.8 | 54.8 | 6.6 |
| ECM yield, kg/d | 37.2 | 31.0 | 55.1 | 6.7 |
| CH₄, g/d | 8304 | 5392 | 11,190 | 1151 |
| CH₄, DMI, g/kg | 359.4 | 227.1 | 492.6 | 48.3 |
| CH₄, N/D, g/kg | 61.1 | 41.1 | 86.6 | 9.3 |
| CH₄, E/ECM, g/kg | 229.5 | 149.8 | 455.0 | 48.1 |
| Total CO₂, g/d | 19,201 | 14,412 | 24,145 | 2004 |
| Total CO₂, DMI, g/kg | 831.5 | 575.2 | 976.7 | 84.1 |
| Total CO₂, E/ECM, g/kg | 141.3 | 101.1 | 183.5 | 17.3 |
| Total CO₂/E, g/kg | 531.1 | 343.3 | 1095.6 | 102.8 |

| a | n, number of observations in the data set |
| b | Feed efficiency, milk yield + dry matter intake (kg/kg), FCM Fat-corrected milk (kg/d) = 0.4 × milk yield (kg/d) + 15 × fat yield (kg/d), ECM Energy-corrected milk (kg/d) = milk yield (kg/d) × [(38.3 × %fat × 10 + 24.2 × %protein × 10 + 16.54 × %lactose × 10 + 20.7) ÷ 3140] [22]. |
solids) percentage and yield, and GHG measurement, including total CO$_2$, total CO$_2$/MW, total CO$_2$/ECM were analyzed using the one-way ANOVA procedure in SAS with repeated measures, according to the following model:

$$Y_i = \mu + T_i + e_i (3)$$

where $Y_i$ is the dependent variable, $\mu$ is the overall mean, $T_i$ is the effect of treatment ($i = 1, 2, 3$), and $e_i$ is the residual error. The statistical significance was defined as $P \leq 0.05$. Differences were considered to be a tendency toward significance at $0.05 < P \leq 0.10$.

**Results**

**Carbon dioxide emissions of lactating Holstein dairy cows**

The data for the overall herd are displayed in Table 2. The mean parity of the cows in the experiment was 2.8 ± 1.0, the mean lactation days was 138 ± 19 d, the mean milk yield was 38.1 ± 6.9 kg/d, and the mean milk yield was 38.1 ± 6.9 kg/d. The CH$_4$ emissions in the experiment were expressed in CO$_2$ equivalent, and the CH$_4$ production (8304 ± 1151 g/d), CH$_4$ yield (359 ± 48 g/kg·DMI), and CH$_4$ intensity (61.1 ± 9.3 g/kg·MW; 229.5 ± 48.1 g/kg·ECM) were calculated. The total CO$_2$ production comprised the CH$_4$ and CO$_2$ production. The total CO$_2$ production of the cows was 19,201 ± 2004 g/d, the total CO$_2$ yield was 831 ± 84 g/kg·DMI, and the total CO$_2$ intensity was 141 ± 17 g/kg·MW and 531 ± 103 g/kg·ECM.

**Carbon dioxide emissions of lactating Holstein dairy cows with different parities**

Using parity as the grouping standard, the cows were divided into SP, TP, and FAP groups (Table 3). There were no significant differences in the metabolic weight, milk production, milk compositions, and total CO$_2$ emissions among the groups ($P > 0.05$). The milk yields of the SP, TP, and FAP groups were 38.5, 37.5, and 37.7 kg/d.

**Carbon dioxide emissions of lactating Holstein dairy cows with different milk yields**

Using milk yield as the grouping standard, the cows were divided into low milk yield (< 34.7 kg/d), medium milk yield (34.7–41.5 kg/d), and high milk yield (> 41.5 kg/d) groups (Table 4). There were no significant differences in the metabolic weight, milk production, milk compositions, and total CO$_2$ emissions among the groups ($P > 0.05$). The milk yields of low, medium, and high milk yields were 29.6 ± 3.8 kg/d, 34.3 ± 3.6 kg/d, and 39.5 ± 2.7 kg/d, respectively. The total CO$_2$ yield was 45.1 ± 10.4 kg/kg·MW and 44.3 ± 8.7 kg/kg·ECM.

**Table 3 Carbon dioxide emissions of lactating Holstein dairy cows with different parities**

| Items$^a$ | SP | TP | FAP | SEM | $P$-value |
|-----------|----|----|-----|-----|-----------|
| Animal description | | | | | |
| Metabolic weight, kg | 133.5 | 140.5 | 138.3 | 2.31 | 0.633 |
| Milk production and composition | | | | | |
| Milk yield, kg/d | 38.5 | 37.5 | 37.7 | 4.43 | 0.379 |
| Milk fat, % | 3.71 | 3.70 | 3.83 | 0.27 | 0.737 |
| Milk protein, % | 3.26 | 3.27 | 3.37 | 0.35 | 0.645 |
| Total milk solids, % | 12.4 | 12.6 | 12.4 | 2.74 | 0.794 |
| FCM yield, kg/d | 36.8 | 35.8 | 36.5 | 4.33 | 0.594 |
| ECM yield, kg/d | 37.6 | 36.5 | 37.1 | 5.29 | 0.633 |
| Carbon dioxide emissions | | | | | |
| Total CO$_2$, g/d | 19,236 | 19,184 | 19,146 | 19.03 | 0.291 |
| Total CO$_2$/MW, g/kg | 144.5 | 137.2 | 139.0 | 4.78 | 0.516 |
| Total CO$_2$/ECM, g/kg | 518.8 | 535.1 | 524.4 | 7.53 | 0.668 |

$^a$SP second parity ($n = 56$), TP third parity ($n = 30$), FAP fourth and above parity ($n = 25$); number of observations in the data set

$^b$FCM Fat-corrected milk (kg/d) = 0.4 × milk yield (kg/d) + 15 × fat yield (kg/d).

**Table 4 Carbon dioxide emissions of lactating Holstein dairy cows with different metabolic weights**

| Items | LMW | MMW | HMW | SEM | $P$-value |
|-------|-----|-----|-----|-----|-----------|
| Metabolic weight, kg | 126.3 | 136.4 | 147.8 | 2.89 | 0.026 |
| Milk production and composition | | | | | |
| Milk yield, kg/d | 38.7 | 38.5 | 36.8 | 2.02 | 0.057 |
| Milk fat, % | 3.66 | 3.73 | 3.81 | 0.26 | 0.062 |
| Milk protein, % | 3.22 | 3.28 | 3.37 | 0.29 | 0.059 |
| Total milk solids, % | 12.4 | 12.3 | 12.7 | 1.03 | 0.826 |
| FCM yield, kg/d | 36.7 | 36.9 | 35.6 | 2.34 | 0.068 |
| ECM yield, kg/d | 37.4 | 37.5 | 36.5 | 2.62 | 0.084 |

**Table 5 Lactation performance of lactating Holstein dairy cows with different milk yields**

| Items | LMY | MMY | HMY | SEM | $P$-value |
|-------|-----|-----|-----|-----|-----------|
| Milk yield, kg/d | 29.6$^a$ | 38.2$^b$ | 45.8$^a$ | 2.08 | 0.024 |
| Milk fat, % | 3.88$^a$ | 3.70$^b$ | 3.65$^a$ | 0.31 | 0.038 |
| Milk protein, % | 3.36$^a$ | 3.30$^{ab}$ | 3.21$^b$ | 0.27 | 0.031 |
| Total milk solids, % | 13.0$^a$ | 12.5$^{ab}$ | 12.0$^b$ | 0.99 | 0.040 |
| FCM yield, kg/d | 28.9$^a$ | 36.4$^b$ | 43.5$^a$ | 2.38 | 0.042 |
| ECM yield, kg/d | 29.6$^a$ | 37.3$^{ab}$ | 44.2$^a$ | 2.48 | 0.039 |

$^a$LMW low metabolic weight (< 131.7, $n = 36$), MMW medium metabolic weight (131.7–141.2, $n = 42$), HMW high metabolic weight (> 141.2, $n = 33$); number of observations in the data set

$^b$FCM Fat-corrected milk (kg/d) = 0.4 × milk yield (kg/d) + 15 × fat yield (kg/d).

$^c$FCM Energy-corrected milk (kg/d) = milk yield (kg/d) × [(38.3 × fat (%) × 10 + 24.2 × protein (%) × 10 + 16.54 × lactose (%) × 10 + 20.7) ÷ 3140]. Total CO$_2$, total CO$_2$/MW, and total CO$_2$/ECM were calculated. The total CO$_2$ yield was 831 ± 84 g/kg·DMI, and the total CO$_2$ intensity was 141 ± 17 g/kg·MW and 531 ± 103 g/kg·ECM.
kg/d, and the total CO₂ production was 19,236, 19,184, and 19,146 g/d, respectively.

Carbon dioxide emissions of lactating Holstein dairy cows with different metabolic weights
Table 4 shows the lactation performance and CO₂ emissions of the cows with different metabolic weights. Compared with the LMW and MMW groups, the HMW group showed the trends of reducing milk, FCM, and ECM yields (0.05 < \( P \) < 0.1); the milk yield decreased by 1.9 and 1.88 kg/d in turns, and there was a tendency to increase the percentages of milk fat and milk protein (0.05 < \( P \) < 0.1). The total CO₂ production of cows among the three groups was 18,996, 19,269, and 19,339 g/d (\( P > 0.05 \)). The HMW group tended to decrease the total CO₂/MW and increase the total CO₂/ECM (0.05 < \( P \) < 0.1).

Carbon dioxide emissions of lactating Holstein dairy cows with different milk yields
The milk production, milk composition, and CO₂ emissions of cows with different milk yields are shown in Table 5, Fig. 1, and Table S1. Milk fat, milk protein, and total milk solids percentages of cows in the HMY group were significantly lower than those in the LMY group (\( P < 0.05 \)). FCM and ECM yields were proportional to the milk yield of the cows (\( P < 0.05 \)). The total CO₂/MW of cows in the MMY and HMY groups were significantly higher than those in the LMY group (\( P < 0.05 \)). Milk yield of cows had a significant positive relationship with total CO₂ production and a negative relationship with total CO₂/ECM (\( P < 0.05 \)). The total CO₂ production of the three groups was 18,033, 19,364, and 20,048 g/d, with the HMY group was 11% higher than the LMY group; however, the total CO₂/ECM of the LMY group was 36% higher than that of the HMY group.

Carbon dioxide emissions of lactating Holstein dairy cows with different milk fat yields
The cows were divided into LMFY, MMFY, and HMFY groups (Table 6, Fig. 2, and Table S2). There were

Table 6 Lactation performance of lactating Holstein dairy cows with different milk fat yields

| Items          | LMFY | MMFY | HMFY | SEM | \( P \)-value |
|----------------|------|------|------|-----|-------------|
| Milk fat yield, g/d | 1138c | 1435b | 1732a | 15.38 | 0.029 |
| Milk yield, kg/d    | 32.1c | 39.1b | 44.2a | 2.01 | 0.038 |
| Milk fat, %         | 3.59c | 3.71b | 3.94a | 0.27 | 0.041 |
| Milk protein, %     | 3.18c | 3.31b | 3.41a | 0.32 | 0.048 |
| Total milk solids, %| 12.6 | 12.3 | 12.6 | 0.93 | 0.863 |
| FCM yield, kg/d     | 29.9c | 37.2b | 43.6a | 2.79 | 0.036 |
| ECM yield, kg/d     | 30.5c | 37.8b | 44.7a | 2.95 | 0.041 |

\(^1\)LMFY low milk fat yield (< 1278, \( n = 39 \)), MMFY medium milk fat yield (1278–1550, \( n = 41 \)), HMFY high milk fat yield (> 1550, \( n = 31 \)), \( n \) number of observations in the data set

\(^2\)FCM Fat-corrected milk (kg/d) = 0.4 × milk yield (kg/d) + 15 × fat yield (kg/d).

\(^3\)ECM Energy-corrected milk (kg/d) = milk yield (kg/d) × [(38.3 × fat (%)) × 10 + 24.2 × protein (%)) × 10 + 16.54 × lactose (%)) × 10 + 20.7] ÷ 3140

\(^{**}\)Means in the same row with different superscripts are significantly different (\( P < 0.05 \))
significant differences in the milk yield, FCM yield, ECM yield, milk fat percentage, and milk protein percentage \((P < 0.05)\), which were positively correlated with milk fat yield. However, there was no difference in the total milk solids percentage among the groups \((P > 0.05)\). The total CO\(_2\) and total CO\(_2\)/MW of the MMFY and HMFY groups were significantly higher than those of the LMFY group \((P < 0.05)\), but there was no difference between the MMFY and HMFY groups \((P > 0.05)\). The total CO\(_2\) levels of the three groups were 17,884, 19,751, and 20,132 g/d, respectively. There were significant differences in total CO\(_2\)/ECM among the three groups \((P < 0.05)\), total CO\(_2\)/ECM decreased with increasing milk fat yield. The total CO\(_2\)/ECM of the MMFY and HMFY groups was 76.1 g/kg and 146.7 g/kg lower than that of the LMFY group, respectively.

**Carbon dioxide emissions of lactating Holstein dairy cows with different milk protein yields**

The cows were separated into three groups based on the milk protein yield (Table 7, Fig. 3, and Table S3). There were significant differences in the milk yield, FCM yield, ECM yield, milk fat percentage \((P < 0.05)\); these values increased with an increase in the milk protein yield. The milk protein percentages of MMPY and HMPY groups were significantly higher than that of the LMPY group \((P < 0.05)\), but there was no difference between MMPY and HMPY groups \((P > 0.05)\). In addition, there was no difference in the total milk solids percentage among the three groups \((P > 0.05)\). The total CO\(_2\) and total CO\(_2\)/MW of the MMPY and HMPY groups were significantly higher than those of the LMPY group \((P < 0.05)\). The total CO\(_2\)/ECM of the three groups was 612.2, 524.4, and 461.5 g/kg, respectively. There were significant differences in the total CO\(_2\)/ECM among the three groups \((P < 0.05)\), the LMPY group was the highest, the HMPY group was the lowest, and the MMPY group was in the middle.

**Table 7** Lactation performance of lactating Holstein dairy cows with different milk protein yields

| Items\(^2\) | LMPY | MMPY | HMPY | SEM  | \(P\)-value |
|------------|------|------|------|------|-------------|
| Milk protein yield, g/d | 983\(^a\) | 1254\(^b\) | 1490\(^a\) | 16.7 | 0.024       |
| Milk yield, kg/d | 31.6\(^a\) | 38.1\(^b\) | 44.2\(^a\) | 2.39 | 0.041       |
| Milk fat, % | 3.65\(^a\) | 3.71\(^b\) | 3.83\(^a\) | 0.31 | 0.039       |
| Milk protein, % | 3.16\(^b\) | 3.31\(^ab\) | 3.39\(^a\) | 0.28 | 0.031       |
| Total milk solids, % | 11.8 | 12.5 | 12.3 | 0.79 | 0.762       |
| FCM yield, kg/d | 29.7\(^a\) | 36.3\(^b\) | 42.9\(^a\) | 2.98 | 0.039       |
| ECM yield, kg/d | 30.1\(^c\) | 37.1\(^b\) | 43.9\(^a\) | 3.65 | 0.025       |

\(^{1}\)LMPY low milk protein yield (< 1130, \(n = 35\)), MMPY medium milk protein yield (1130–1364, \(n = 39\)), HMPY high milk protein yield (> 1364, \(n = 37\)), \(n\) number of observations in the data set

\(^{2}\)FCM Fat-corrected milk (kg/d) = 0.4 \times milk yield (kg/d) + 15 \times milk fat yield (kg/d).

ECM Energy-corrected milk (kg/d) = milk yield (kg/d) \times \{38.3 \times fat (%) \times 10 + 24.2 \times protein (%) \times 10 + 16.54 \times lactose (%) \times 10 + 20.7\} / 3140

\(^{*}\)Means in the same row with different superscripts are significantly different \((P < 0.05)\)
Carbon dioxide emissions of lactating Holstein dairy cows with different total milk solids yields

Milk, FCM, and ECM yields increased with the increase of total milk solids yield, and there were significant differences among the three groups \((P < 0.05, \text{Table 8})\). However, there were no significant differences in the milk fat, milk protein, and total milk solids percentages among the three groups \((P > 0.05)\). There were significant differences in the total CO₂ production and total CO₂/ECM of cows between each group \((P < 0.05)\), and total milk solids had a positive relationship with total CO₂ production and a negative relationship with the total CO₂/ECM (Fig. 4, and Table S4). The total CO₂/MW of cows in the MTMSY and HTMSY groups was significantly higher than that in the LTMSY group \((P < 0.05)\), and there was no difference between the MTMSY and HTMSY groups \((P > 0.05)\).

**Table 8** Lactation performance of lactating Holstein dairy cows with different total milk solids yields

| Items                        | LTMSY | MTMSY | HTMSY | SEM  | \(P\)-value |
|------------------------------|-------|-------|-------|------|-------------|
| Total milk solid yield, g/d  | 3789  | 4714  | 5512  | 20.3 | 0.031       |
| Milk yield, kg/d             | 31.0  | 37.6  | 44.5  | 2.36 | 0.021       |
| Milk fat, %                  | 3.74  | 3.73  | 3.73  | 0.20 | 0.916       |
| Milk protein, %              | 3.27  | 3.31  | 3.29  | 0.22 | 0.871       |
| Total milk solids, %         | 12.4  | 12.6  | 12.4  | 0.86 | 0.893       |
| FCM yield, kg/d              | 29.5  | 36.0  | 42.7  | 2.34 | 0.036       |
| ECM yield, kg/d              | 29.9  | 36.9  | 43.6  | 2.35 | 0.041       |

\(^1\)LTMSY low total milk solids yield (< 4325, \(n = 33\)); MTMSY medium total milk solids yield (4325–5115, \(n = 39\)); HTMSY high total milk solids yield (> 5115, \(n = 39\)); \(n\), number of observations in the data set

\(^2\)FCM Fat-corrected milk (kg/d) = 0.4 \times \text{milk yield (kg/d)} + 15 \times \text{fat yield (kg/d)}.

\(^3\)ECM Energy-corrected milk (kg/d) = \text{milk yield (kg/d)} \times (38.3 \times \text{fat (\%)} \times 10 + 24.2 \times \text{protein (\%)} \times 10 + 16.54 \times \text{lactose (\%)} \times 10 + 20.7) \div 3140.

\(^a\)Means in the same row with different superscripts are significantly different \((P < 0.05)\)

**Discussion**

Carbon dioxide emissions of lactating Holstein dairy cows

The data of this experiment were obtained under the normal feeding conditions of the dairy farm, so it had stronger reliability and representativeness [23]. This analysis contributed to further understanding of the GHG emission characteristics of Chinese lactating Holstein dairy cows.

In terms of GHG emissions, the CH₄ production was 8304 g/d (expressed as CO₂ equivalents), CH₄ yield was 359 g/kg-DMI (CO₂-eq), CH₄ intensity was 229.5 g/kg-ECM (CO₂-eq), and total CO₂ production was 19,201 g/d. Niu et al. [24] summarized 2566 data points from Europe, the United States, and Australia. The CH₄ production of dairy cows was 9225 g/d (CO₂-eq), CH₄ yield was 502.5 g/kg-DMI (CO₂-eq), and CH₄ intensity...
was 337.5 g/kg·ECM (CO₂−eq). The results of Niu et al. [24] were higher than the results of the present experiment, probably because the Chinese Holstein lactating dairy cow diets were relatively higher in the concentrate-to-forage ratio, such as lower NDF (32.8% vs. 35.4%) and higher EE (5.5% vs. 3.5%) content decreased CH₄ emissions [24–26]. Or the CH₄ measurement method and the characteristics of the cows were different, Niu’s data were derived from Holstein, Ayrshire, Jersey, Brown Swiss, Simmental, and crossbred dairy cows measured using respiration chambers, the GreenFeed system, and sulfur hexafluoride (SF₆) tracer technique [24]. Therefore, a test with the same measurement methods and similar cow characteristics was carried out. The lactating Holstein dairy cows had an average milk yield of 39.8 kg/d, DMI of 25.3 kg/d, DIM of 115 d, and body weight of 624 kg at the beginning of the experiment [27]. The results of Oh et al. [27] were similar to those of the present study, showing that the CH₄ production of cows was 8425 g/d (CO₂−eq), CH₄ yield was 332.5 g/kg·DMI (CO₂−eq), CH₄ intensity was 231.5 g/kg·ECM (CO₂−eq), and total CO₂ production was 20,644 g/d. The present experiment showed that each Holstein lactating dairy cow emitted 7008 kg of the total CO₂ per year in East China.

DMI is the primary factor affecting the emission of CH₄ from the cows [7]. As a result, utilizing DMI to predict CH₄ emissions is more accurate, but the data are more difficult to obtain. In addition, the different types of diets also have an impact on CH₄ emissions. Therefore, the present study focused on Holstein lactating dairy cow’s variables, such as parity, weight, milk production, and milk composition; and their impact on exhaled GHG emissions was studied.

**Carbon dioxide emissions of lactating Holstein dairy cows with different parities**

Parity is an essential physiological indicator in cows. The weight of the primiparous cows was lower, and the nutrients ingested by them were distributed to the body for growth, causing the metabolism, DMI, milk production, and fertility of primiparous cows to be different from those of the multiparous cows [28–30]. Only multiparous lactating Holstein dairy cows were chosen as experimental animals to eliminate this influencing factor. The present experiment showed that there was no difference in the metabolic body weight among lactating Holstein dairy cows of various parities, indicating that the physiological structure of cows matured after the second parity. In addition, there were no differences in the milk yield or milk component concentration among the different groups. Similar results have been reported in other studies. The milk yield of first parity was the lowest, but there were no differences among the second, third, and...
fourth parities [31]. The parity number also did not affect the contents of milk fat and protein during early lactation [31]. It is generally believed that after a cow reached a certain age, the lactation performance would decrease with the increase in parity [32]. This problem did not appear in the present experiment probably because of the proper daily feeding on the dairy farm, proper management of the herd, and the small number of cows with more than fourth parity.

Grandl et al. [32] showed that the CH4 emissions of the cows peaked during the second to third lactation period until CH4 production, CH4 yield, and CH4 intensity were low at about 6.5 years of age. There were no significant differences in the total CO2 production, total CO2/MW, and total CO2/ECM among the groups in this experiment. Chewing efficiency resulted in fiber degradation, which was the greatest of medium-aged cows [33]. Methane emissions had the concomitant relationship with fiber digestibility, so lower in young and old cows. Only a few cows over 6.5 years old were included in the present experiment, perhaps because it is very common to eliminate older cows in pursuit of higher feeding efficiency in Chinese dairy farms. Therefore, parity was not a factor affecting CO2 emissions from lactating Holstein dairy cows in the present experiment.

**Carbon dioxide emissions of lactating Holstein dairy cows with different metabolic weights**

Contrary to the results of this experiment, it is generally believed that although the relationship between body weight and milk production is not very strong, the cows with high milk production tend to be larger [34]. Previous studies have shown that the body condition score directly affected by the body weight was negatively correlated with milk production, and negatively correlated with reproductive performance [35, 36]. The cow body weight seemed to be positively correlated with the incidence of metritis and milk somatic cell score [37, 38]. Therefore, excessive metabolic body weight would be detrimental to the lactation performance of lactating Holstein dairy cows.

According to Blaxter and Czerkawski [39], reducing CH4 production from the rumen provides more metabolizable energy utilization for the growth of body tissues. Hristov et al. [40] showed that the reduction in CH4 emission from Holstein cows significantly increased the rate of weight gain. Previous studies illustrated how a reduction in dietary GE loss, such as CH4, can increase the energy available for production purposes, that is, improve lactose and protein synthesis in milk, or restore weight loss during early lactation [40]. Although there was no significant difference in total CO2 production between cows with different metabolic weights, the total CO2 production of low metabolic weight cows were quantitatively lower than that of high metabolic weight cows in current experiment. Van Zijderfeldt et al. [41] concluded that weight gain did not always improve when the CH4 production in dairy cows was suppressed. For example, if the cows’ weight loss in early lactation has been restored, then the weight of middle lactation cows would remain stable.

Some studies have reported a negligible relationship between live weight and CH4 emissions, but lighter animals ate less and therefore produced less total gas emissions [42]. In contrast to these studies, there was no difference in the total CO2 production among the different groups in the present test. The CH4 emissions of dairy cows and the digestibility of dietary fiber showed similar changes, according to Grandl et al. [32]. It was speculated that although the cows with high metabolic weight had high feed intake, their dietary digestibility was low, therefore they did not affect enteric gas emissions. There was no doubt that the higher the metabolic weight, the lower the total CO2/MW. The numerical order of the total CO2/MW was HMW group < MMW group < LMY group. The milk yield of cows did not increase with an increase in the metabolic weight in this study. Therefore, the order of the size of the total CO2/ECM was the opposite to that of the total CO2/MW. Higher-weight cows had a negative impact on lactation performance and GHG reduction.

**Carbon dioxide emissions of lactating Holstein dairy cows with different milk yields**

Bedô et al. [43] showed that milk component percentage was negatively correlated with milk yield of dairy cows. This is easy to understand: the higher the milk production, the lower the concentration of milk components [44]. The present experiment also demonstrated that the proportions of milk fat, milk protein, and total milk solids decreased with the increase of milk production.

Reducing GHG emissions is one of the key goals of dairy industry [12]. A previous study demonstrated a significant positive correlation between milk yields and CH4 emissions [45]. Gerber et al. [46] showed that higher milk yields result in higher CO2, CH4 and nitrous oxide emissions per cow. The result is in line with previous studies, which the total CO2 production of the HMY group was significantly higher than that of the LMY group by 2015 g/d in the current study. From the perspective of total CO2 production, the dairy cows with high milk yield did not seem to be conducive to the mitigation of total CO2. However, emissions per unit of animal products reflect the accuracy of management practices on the composite of feed intake, GHG emissions, and animal productivity [47]. Evaluating the total CO2 emission capacity of lactating Holstein dairy cows should be based on the CO2 production relative to ECM.
because the ultimate goal of the dairy farming industry was to obtain milk.

It is estimated that the rapid growth of the global population, combined with the improvements in global living standards, would lead to a 48% increase in global demand for dairy products between 2005 and 2050 [48]. As per the goal of the Chinese government’s dairy industry development, China is estimated to produce 45 million tons of milk by 2025, showing an increase of 40% over 2019 [49]. This would expand the dairy industry and increase the number of dairy cows. Although China is the world’s third-largest milk producer, low-productivity milk production has a greater impact on the environment compared to that from developed countries [50, 51]. The present experiment showed that the total CO₂/ECM of the HMY group was significantly lower by 167 g/kg than that of the LMY group. In other words, the higher the milk yield of lactating Holstein dairy cows, the lower would be the total CO₂ production per unit of ECM. Similarly, the study discovered that as milk production increases, GHG emissions per kg of fat and protein corrected milk decrease significantly [46]. In 2019, the average milk production of dairy cows in China was only 5647 kg/head, which is lower than that of Europe and New Zealand, and there is still much room for improvement in milk production [56]. Therefore, development goals should be formulated for the dairy industry, by increasing the milk production of lactating Holstein dairy cows. It is possible to feed fewer cows to obtain more milk while reduce GHG emissions.

**Carbon dioxide emissions of lactating Holstein dairy cows with different milk component yields**

Milk fat percentage is not only an important index for evaluating milk quality but also for evaluating the lactation performance and mammary gland health of dairy cows. There were significant differences in the milk production and milk component percentage between the different milk fat yield groups, and that of the HMFY group was higher than that of the LMFY group, except for the total milk solids percentage. Generally, the concentration of milk component decreased with the increase of milk yield due to dilution effect [52]. However, the results of this experiment revealed that milk fat yield was higher only when the milk yield and milk fat percentage were both high. The reason for there being no differences between the total milk solids percentages might be because the milk fat and the milk protein percentages were positively correlated, while the lactose percentage was negatively correlated with them, which ultimately balanced the total milk solids percentage among the groups [53].

The total CO₂ production in the HMFY and MMFY groups was higher than that in the LMFY group. This is probably because a higher milk yield would require higher feed intake, digestion, absorption, and metabolism, and DMI is a major driver of enteric CH₄ emission [7], which would in turn produce more CH₄ and CO₂ [7]. There was no difference between the HMFY and MMFY groups, indicating that the digestive and metabolic functions of the animals had an upper limit and could not continue to increase. The present study has concluded that the weight of dairy cows did not increase because of the increase in milk yield, so the total CO₂/MW of MMFY and HMFY groups was significantly higher than that of the LMFY group. However, the total CO₂/ECM of cows with a high milk fat yield was lower than that of cows with low milk fat yield. The total CO₂/ECM of the MMFY and HMFY groups was 13% and 24% lower than that of the LMFY group. From the perspective of animal products, the cows with higher milk fat yield are more conducive to reducing GHG emissions.

 Protein is an important nutrient component of milk that can provide people with high-quality functional proteins, and its yield is closely related to economic benefits. We have been trying to improve the milk protein yield of lactating Holstein dairy cows through herd management, nutrition, and genetics [8]. There were significant differences in the milk yield, milk fat and milk protein percentages among the groups, and the HMPY group was higher than the LMPY group. The present experiment showed that milk protein percentage decreased with the increase of milk yield. However, it can be seen from the data of the cows with various milk protein yields that the milk protein yield was higher only when the milk yield and milk protein percentage were both high. These results were consistent with those reported by Xue et al. [8]. In this experiment, the total CO₂ production and total CO₂/MW of cows with higher milk protein yield were higher than those of cows with lower milk protein yield. However, the GHG emissions of cows with higher milk protein yield were lower than those of cows with lower milk protein yield, when the emissions expressed as per kg of ECM. The total CO₂/ECM of the MMFY and HMPY groups was 87.8 and 150.7 g/kg lower than that of the LMPY group, respectively. The rationale for this difference was the same as the difference in milk fat yield groups, and the cows with higher milk protein yields are more conducive to reducing GHG emissions.

In addition to the two major nutrients of milk fat and milk protein, milk also contains lactose, vitamins, and minerals; therefore, total milk solids is also an important indicator of milk quality. Milk yield was positively correlated with total milk solids yield, on the other hand, the concentration of milk component in the three groups did not differ in the current study. Cows with higher
total milk solids yield had higher total CO$_2$ production and total CO$_2$/MW than these with lower total milk solids yield. However, the total CO$_2$/ECM decreased with the increase of total milk solids yield of dairy cows. The number of cows and heifers required for the same total milk solids yield under different lactation performance conditions varied greatly [54]. In the dairy industry, the total milk solids yield is positively correlated with CH$_4$ emissions [55], while CH$_4$ intensity (per kg of milk production) decreases as milk yield improves [54]. In line with these earlier studies, with the increase in total milk solids yield of lactating Holstein dairy cows, the total CO$_2$ intensity (CO$_2$ per kg of ECM yield) decreased in this test. The total CO$_2$/ECM of LTMSY, MTMSY, and HTMSY groups was 614.3, 530.0, and 461.9 g/kg, respectively. However, with the improvement of the standards of living of the people of China, the development of the milk industry would be promoted. Therefore, it is important to determine the methods and strategies to find a balance between minimizing environmental impact and increasing animal productivity to meet the demands of the world population for animal protein. It is an effective carbon emission reduction measure to reduce the number of cows and the total CO$_2$ intensity by increasing the milk component yield of lactating Holstein dairy cows.

**Conclusions**

This study demonstrates that total CO$_2$ emissions from lactating Holstein dairy cows in East China averaged 19,201 ± 2004 g/d, 831 ± 84 g/kg·DMI, 141 ± 17 g/kg·BW, and 531.1 ± 103 g/kg·ECM, respectively. Lactating Holstein dairy cows with low milk yield, milk fat yield, milk protein yield, and total milk solids yield produced less total CO$_2$, but their total CO$_2$ production per kg of ECM was higher. Therefore, it was concluded that selecting lactating Holstein dairy cows with less total CO$_2$ production would probably reduce production efficiency and significantly increase the production cost of the dairy products. Low total CO$_2$ intensity (total CO$_2$/ECM) cows demonstrated higher efficiency in terms of energy utilization efficiency, while produced more milk. To promote low carbon development, more research with lactating Holstein dairy cows from different geographical locations, physiological stages, production systems in China is needed to establish regional or national GHG inventories as well as develop mitigation approaches to dairy production regimes.

**Abbreviations**

ADF: Acid detergent fiber; Ca: Calcium; CH$_4$: Methane; CO$_2$: Carbon dioxide; CP: Crude protein; DM: Dry matter; DMI: Dry matter intake; ECM: Energy-corrected milk; EE: Ether extract; FAP: Fourth and above parity; FCM: Fat-corrected milk; GE: Gross energy; GHG: Greenhouse gas; HMFY: High milk fat yield; HMPY: High milk protein yield; HTMSY: High total milk solids yield; HMW: High metabolic weight; HMY: High yield; LMFY: Low milk fat yield; LMPY: Low milk protein yield; LTMSY: Low total milk solids yield; LMW: Low metabolic weight; LMY: Low yield; MMPY: Medium milk protein yield; MMW: Medium metabolic weight; MMFY: Medium milk fat yield; MMTSY: Medium total milk solids yield; MTMSY: Medium total milk solids yield produced less total CO$_2$/ECM, but their total CO$_2$ production per kg of ECM was higher. Therefore, it was concluded that selecting lactating Holstein dairy cows with different milk protein yields.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s40104-022-00721-3.

**Acknowledgments**

The authors thank colleagues at the Institute of Feed Research and Yinxiangweiye Group Co., Ltd., for providing kind assistance in the animal experiments, sample processing, and data collection. The authors would like to thank reviewers for their kind suggestions and comments on this work.

**Authors’ contributions**

Formal analysis, PJ, ZHL, and QL; methodology, YT, FDL, LFD, and QYD; project administration, LFD and QYD; writing (original draft) PJ, writing (review and editing) PJ, LFD, and QYD. The author(s) read and approved the final manuscript.

**Funding**

This study was funded by the Central Public-interest Scientific Institution Basal Research Fund of Chinese Academy of Agricultural Sciences (No. Y2022GH12) and the Agricultural Science and Technology Innovation Program (CAAS-ASTIP-2017-FRI-04).

**Availability of data and materials**

All data involved in this study are included in this article and its supplementary files.

**Declarations**

**Ethics approval and consent to participate**

The animals involved in this experiment were cared for according to the guidelines of the Animal Ethics Committee of the Chinese Academy of Agricultural Sciences (protocol number 019–2018). The committee reviewed and approved the experiments and procedures involving the use of animals.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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37. Markusfeld O, Ezra E. Body measurements, metritis, and postpartum performance of first lactation cows. J Dairy Sci. 1993;76(12):3771–7. https://doi.org/10.3168/jds.0022-0302(93)77720-6.

38. Berry DP, Lee JM, Macdonald KA, Stafford K, Matthews L, Roche JR. Associations among body condition score, body weight, somatic cell count, and clinical mastitis in seasonally calving dairy cattle. J Dairy Sci. 2007;90(2):637–48. https://doi.org/10.3168/jds.0022-0302(07)71546-1.

39. Blaxter KL, Czerkawski J. Modifications of the methane production of the sheep by supplementation of its diet. J Sci Food Agric. 1996;17(9):417–21. https://doi.org/10.1002/jsfa.2740170907.

40. Hristov AN, Oh J, Giallongo F, Frederick TW, Harper MT, Weeks HL, et al. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proc Natl Acad Sci U S A. 2015;112(34):10663–8. https://doi.org/10.1073/pnas.1515515112.

41. van Zijderveld SM, Gerrits WJ, Dijkstra J, Newbold JR, Hulshof RBA, Perdok HB. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. J Dairy Sci. 2011;94(8):4028–38. https://doi.org/10.3168/jds.2011-4236.

42. Department for Environment Food and Rural Affairs, 2014. Evidence final report project: Improvements to the national inventory: methane. Project AC0115 improvements to the national inventory: Methane. London: Department for Environment, Food and Rural Affairs; 2014. http://refhub.elsevier.com/S1751-7311(21)00168-3/h0080.

43. Bedö S, Nikodemusz E, Percsich K, Bárdos L. Variations in the milk yield and milk composition of dairy cows during lactation. Acta Vet Hung. 1995;43(1):163–71.

44. Goetsch AL, Zeng SS, Gipson TA. Factors affecting goat milk production and quality. Small Rumin Res. 2011;101(1–3):55–63. https://doi.org/10.1016/j.smr.2011.09.025.

45. Dehareng F, Delfosse C, Froidmont E, Soyeurt H, Martin C, Gengler N, et al. Potential use of milk mid-infrared spectra to predict individual methane emission of dairy cows. Animal. 2012;6(10):1694–701. https://doi.org/10.1017/S1751731112000456.

46. Gerber P, Vellinga T, Opio C, Steinfeld H. Productivity gains and greenhouse gas emissions intensity in dairy systems. Livest Sci. 2011;139(1–2):100–8. https://doi.org/10.1016/j.livsci.2011.03.012.

47. Greenhouse gas emissions from dairy sector. Rome: Food and Agriculture Organization of the United Nations; 2010. http://faostat.fao.org/docrep/012/k7930e/k7930e00.pdf.

48. Alexandratos N, Bruinsma J. World Agriculture Towards 2030/2050: the 2012 revision (No. 12-03). ESA Working paper. Rome: FAO; 2012.

49. MOA. Opinions on further promoting the development of dairy industry. Beijing: Ministry of Agriculture and Rural Affairs of the People’s Republic of China; 2018. http://www.moa.gov.cn/nybgb/2019/201901/201905/t20190503_6288218.htm.

50. Bai Z, Ma L, Oenema O, Chen Q, Zhang F. Nitrogen and phosphorus use efficiencies in dairy production in China. J Environ Qual. 2013;42(4):990–1001. https://doi.org/10.2134/jeq2012.0464.

51. Eshel G, Shepon A, Makov T, Milo R. Land, irrigation water, greenhouse gas, and reactive nitrogen burdens of meat, eggs, and dairy production in the United States. Proc Natl Acad Sci U S A. 2014;111(3):11996–2001. https://doi.org/10.1073/pnas.1402183111.

52. Ramin M, Fant P, Huhtanen P. The effects of gradual replacement of barley with oats on enteric methane emissions, rumen fermentation, milk production, and energy utilization in dairy cows. J Dairy Sci. 2021;104(5):5617–30. https://doi.org/10.3168/jds.2020-19644.

53. Rook JAF, Camping RC. Effect of stage and number of lactation on the yield and composition of cow’s milk. J Dairy Res. 1965;32(01):45–55. https://doi.org/10.1017/S0022029900018367.

54. Dall-Orsolaletta AC, Leurent-Colette S, Launay F, Ribeiro-Filho HMN, Delaby L. A quantitative description of the effect of breed, first calving age and feeding strategy on dairy systems enteric methane emission. Livest Sci. 2019;224:68–75. https://doi.org/10.1016/j.livsci.2019.04.015.

55. Zhang X, Amer PR, Jenkins GM, Sise JA, Santos B, Quinton C. Prediction of effects of dairy selection indexes on methane emissions. J Dairy Sci. 2019;102(12):11153–68. https://doi.org/10.3168/jds.2019-16943.

56. FAOSTAT. FAO statistical databases. Rome: Food and Agriculture Organization of the United Nations; 2019. http://www.fao.org/faostat/en/#home.

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