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Are dairy cows with a more reactive temperament less efficient in energetic metabolism and do they produce more enteric methane?

M.G. Marçal-Pedroza [a,b], M.M. Campos [c], J.P. Sacramento [d], L.G.R. Pereira [c], F.S. Machado [c], T.R. Tomich [c], M.J.R. Paranhos da Costa [e], A.C. Sant'Anna [a,f,*]

[a] Nucleus of Studies and Research in Ethology and Animal Welfare (NEBEA), Department of Zoology, Institute of Biological Sciences, Federal University of Juiz de Fora, 36.036-330 Juiz de Fora, Minas Gerais, Brazil
[b] Postgraduation Program in Biodiversity and Nature Conservation, Federal University of Juiz de Fora, 36.036-330 Juiz de Fora, Minas Gerais, Brazil
[c] Brazilian Agricultural Research Corporation, Embrapa Dairy Cattle, 36038-330 Juiz de Fora, Minas Gerais, Brazil
[d] Department of Bioengineering, Federal University of São João Del Rei, São João del-Rei, Minas Gerais, Brazil
[e] Group of Studies and Research in Ethology and Animal Ecology, Department of Animal Science, Faculty of Agricultural and Veterinarian Sciences, São Paulo State University (UNESP), 14.884-900 Jaboticabal, São Paulo, Brazil
[f] National Council for Scientific and Technological Development, CNPq Researcher, Brazil

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ABSTRACT

It remains unknown whether dairy cows with more reactive temperament produce more enteric methane (**CH_4**) and are less bioenergetically efficient than the calmer ones. The objectives of this study were (a) to evaluate the relationship between cattle temperament assessed by traditionally used tests with energetic metabolism and enteric CH_4 emissions by crossbred dairy cows; (b) to assess how cows' restlessness in respiration chambers affects energetic metabolism and enteric CH_4 emissions. Temperament indicators were evaluated for 28 primiparous F1 Holstein-Gyr cows tested singly in the handling corral (entrance time, crush score, flight speed, and flight distance) and during milking (steps, kicks, defecation, rumination, and kick the milking cluster off). Cows' behaviors within respiration chambers were also recorded for each individual kept singly. Digestibility and calorimetry trials were performed to obtain energy partitioning and CH_4 measures. Cows with more reactive temperament in milking (the ones that kicked the milking cluster off more frequently) spent 25.24% less net energy on lactation (P = 0.04) and emitted 36.77% more enteric CH_4/kg of milk (P = 0.03). Furthermore, cows that showed a higher frequency of rumination at milking parlor allocated 57.93% more net energy for milk production (P < 0.01), spent 50.00% more metabolizable energy for milk production (P < 0.01) and 37.10% less CH_4/kg of milk (P = 0.04). Regarding the handling temperament, most reactive cows according to flight speed, lost 29.16% less energy as urine (P = 0.05) and tended to have 14.30% more enteric CH_4 production (P = 0.08), as well as cows with a lower entrance time (most reactive) that also lost 13.29% more energy as enteric CH_4 (P = 0.04). Temperament and restless behavior of Holstein-Gyr cows were related to metabolic efficiency and enteric CH_4 emissions. Cows' reactivity and rumination in the milking parlor, in addition to flight speed and entrance time in the squeeze chute during handling in the corral, could be useful measures to predict animals more prone to metabolic inefficiency, which could negatively affect the sustainability of dairy systems.

Implications

Livestock production plays an important role in the greenhouse gas emissions, part of them comes from enteric methane emissions of cattle. In this study, we assessed the effects of cows' behavior on their energetic metabolism and enteric methane emissions. We have found that environmental consequences might arise from the inefficient feeding resource use, increasing methane emissions by temperamental and reactive cattle. We recommend improving temperament throughout animal breeding and good practices of cattle handling as viable strategies for attaining a more sustainable dairy production.

* Corresponding author at: Nucleus of Studies and Research in Ethology and Animal Welfare (NEBEA), Department of Zoology, Institute of Biological Sciences, Federal University of Juiz de Fora, 36.036-330 Juiz de Fora, Minas Gerais, Brazil.
E-mail address: aline.santanna@ufjf.edu.br (A.C. Sant'Anna).

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Introduction

Sustainable livestock production has been a theme of debates in the international scene, raising new challenges for the stakeholders of farm animal production chains (van Dijk et al., 2019). Public opinion has shown an increasing interest in the acquisition of high-quality animal products. It includes the requirement of information about the products' origin and the productive processes, comprising issues related to their impacts on animal welfare and environment (Risius and Hamm, 2017). This is related to a growing global demand for an ethical and sustainable way to develop the economic activities, including the livestock production. The concept of "One Welfare" seems to be a useful guide to achieve this since it proposes that the activities that affect (positively or negatively) animal welfare, human wellbeing, biodiversity, and environmental conservation are closely connected and are mutually dependent on each other (García et al., 2016; Tarazona et al., 2019).

In this context, one of the challenges is the efficient use of resources and the mitigation of greenhouse gas emissions by livestock (Herrero et al., 2016). Enteric methane (**CH_4**) is one of the greenhouse gasses produced during the digestive process of ruminants by the action of anaerobic microorganisms that colonize the rumen, through fermentation of plant carbohydrate (Beauchemin et al., 2008). In Brazil, estimates pointed out that ruminants' enteric fermentation was responsible for 11 352 (t) of methane produced in 2017, and the dairy industry contributed with 0.33 L of methane/kg of milk in the country (SEEG, 2018).

There is a variation in the amount of CH_4 emission by ruminants; thus, it is important to understand which factors affect the enteric CH_4 production by these animals. For example, quality of the diet (Cottle et al., 2011), level of dry matter intake (Dini et al., 2019), environmental temperature (Yadav et al., 2016) were reported to be associated with CH_4 emissions. Thus, some possible alternatives for CH_4 mitigation have been investigated, most of them including nutritional strategies (Haque, 2018), besides other alternatives, such as intensification of the productive systems (de Vries et al., 2015). Despite considerable recent progress in the nutritional field, several other factors related to animal physiology may contribute to their bioenergetic efficiency and reduction of greenhouse gas emissions (Ornelas et al., 2019), which still deserve to be better understood.

There is some evidence showing that physiological and behavioral responses to stress might be associated with a higher enteric CH_4 production (Yadav et al., 2016; Llonch et al., 2018) and lower productivity in dairy cows (Hedlund and Løvlie, 2015). The emissions of enteric CH_4 represent an environmental concern and a source of energetic efficiency reduction due to the loss of gross energy as CH_4 (Johnson and Johnson, 1995). The energy released as CH_4 gas could be allocated for weight gain (in beef cattle) and milk yield (in dairy cattle), ranging from 2% to 12% of the animals' energy intake, depending on the type of diet (Johnson and Johnson, 1995). Thus, strategies for enteric CH_4 mitigation should result in environmental and economic gains, optimizing the use of nutrients.

Temperament had been defined as individual differences in animals' behavioral responses to stressors (Fordyce et al., 1982; Koolhaas et al., 2010). Previous studies have shown that 'nervous' and restless cows produce less milk (Sutherland and Dowling, 2014; Hedlund and Løvlie, 2015); however, the metabolic mechanisms underlying this relationship are poorly understood. One could expect that animals with divergent temperaments would differ in their efficiency to convert the feed energy into milk, i.e., reactive cows could be less efficient than the reactive ones. Thus, cattle temperament could affect the energetic partition, decreasing the energy to milk yield. If reactive cows, in fact, lose a higher percentage of energy through feces, urine, heat production, and CH_4, the temperamental animals may show a more significant impact on the sustainability of the dairy industry. However, these hypotheses still lack empirical support for dairy animals, remaining unknown whether animals with more reactive temperament and restless behavior produce more CH_4 (Llonch et al., 2016) and are less bioenergetically efficient than the calmer ones.

Therefore, the aims of this study were (a) to evaluate the relationships between cattle temperament assessed by traditionally used tests with energetic metabolism and enteric CH_4 emission by Holstein-Gyr dairy cows; (b) to assess how cows' restlessness in the respiration chambers affects energetic metabolism and enteric CH_4 emissions. We hypothesize that individuals with a more reactive temperament and restless in a situation of physical restraint would be metabolically and bioenergetically less efficient than the calmer ones, showing higher enteric CH_4 emission.

Material and methods

Animals and housing conditions

Data were collected from April to November 2017, at the Multi-use Livestock Complex of Bioefficiency and Sustainability of the Brazilian Agricultural Research Corporation, Embrapa (Coronel Pacheco, Minas Gerais, Brazil), with twenty eight primiparous F1 Holstein-Gyr lactating cows, aging 30 ± 1.04 years (mean ± SD) and weighing 568 ± 41.50 kg. Cows were kept in a free-stall barn equipped with an electronic feeding system (AF-1000 Master Gate, Intergado Ltd., Contagem, MG, Brasil) and water troughs (WD-1000, Intergado Ltd., Contagem, Minas Gerais, Brazil). Twice a day, cows were milked in a fishbone milking parlor (2 × 4) (DeLaval, Tumba, Sweden), always by the same two stockpersons. More details about the animals and facilities were previously published in Marçal-Pedroza et al. (2020) that it is part of the same study. Individual daily milk yield data were recorded automatically on the days of the behavioral observations.

Temperament assessment

The cows' temperament was measured based on the cows' behavioral responses to being handled by humans, assessed during milking (i.e., milking temperament) and during handling in the corral (handling temperament). The temperament data used come from data collected in a previous study (Marçal-Pedroza et al., 2020). The milking temperament of the lactating cows was evaluated 45 days after calving, and the subsequent sessions with an average interval of 45 days, performing three sessions along the early lactation period. In each session, data collection was made on three consecutive days, always in the morning milking (a total of nine days of assessment). The following behavioral indicators of cattle temperament were recorded by a previously trained observer, as described in Marçal-Pedroza et al. (2020): number of Steps (STEPS), number of Kicks (KICKS) and the occurrences of behaviors defecation, rumination, and kick the milking cluster off (KOFF), from the time that the milking cluster was attached until its extraction when milking was finished.

The handling temperament was assessed on the last day of each milking evaluation session, in a total of three evaluations in the corral. The following measures were used: Entrance Time (in s), Crush Score, Flight Speed (in m/s), Flight Distance (in m). For the full description of the temperament methods used, please see Marçal-Pedroza et al. (2020).

Whole tract digestibility and energy partitioning

The digestibility assays took place every 45 days throughout all lactation, for a total of six sampling periods. For the digestibility assays, groups of eight cows were transferred to a tie-stall system with individual feeders and water troughs. Individual samplings of feces were collected for five days per group. Total urine was collected on the first two days of the fecal collection. Aliquots of silage, concentrate, and orts were daily collected along the five consecutive days and stored at −20 °C (Supplementary Table S1). The full description of the methods and equations used was included as Supplementary Material S1.

For the calculation of the energy partition, the gross energy intake (**GEI**), daily fecal (Fecal-E, Mcal/d) and urinary (Urine-E, Mcal/d) energy outputs were obtained by multiplying DM intake (**DMI**) and fecal and urinary dry matter excretion with their respective energy contents. Digestible energy intake (**DEI**, Mcal/d) was calculated as the difference between GEI and fecal energy excretion. Metabolizable energy intake (**MEI**, Mcal/d) was derived as the difference between DEI and the sum of Urine-E and CH_4 energy (CH_4-E, Mcal/d), which was assumed to be 45 Kcal/L (Brouwer, 1965). Energy retention was calculated as the difference between MEI and heat production (Heat-E). Heat-E (Kcal/d) was determined based on measurements of O_2 consumption (L/d), CO_2 and CH_4 production (L/d), using the equation of Brouwer (1965). The net energy of lactation (**NEL**) was also obtained based on the feed energy available for milk production after digestive and metabolic losses (in Mcal/kg). The additional measures were also used in the analyses: metabolizable energy/digestible energy (MEI/DEI), metabolizable energy/gross energy (MEI/GEI), energy balance (**EB**), and milk-energy/metabolizable energy (Milk-energy/MEI). These methods were described in Ornelas et al. (2019), carried out under the same conditions and installations of our study.

Respiration measurements

The open-circuit respiration chambers (n = 4) were used to measure gas exchanges. The full description of the chambers system used and its validation was previously published in Machado et al. (2016). Briefly, the net volume of each chamber is 21.10 m^3, containing a 2.26 × 1.26 m pen. The chambers have large double-glazed windows (150 cm high, 150 cm wide) to guarantee visual contact between the animals. Each chamber is fitted with one large back door for animal access and a smaller front door for operator access and feeding. The common gas analysis and data acquisition system were shared by the four chambers (Sable Systems International, Las Vegas, USA). Infrared technology was used to analyze CO_2 and CH_4 concentrations, whereas fuel cell technology was used for O_2. The injection of known volumes of CO_2 and CH_4 in each chamber was used to perform the recovery test of the whole system, using a mass flowmeter (MC-50SLPM-D, Alicat Scientific Inc., Tucson, AZ). The average recovery of the four chambers for CO_2 (mean ± SD) was 87.87 ± 0.04% and for CH_4 was 84. 75 ± 0.07%.

The animals were halter-trained, adapted to handling and went to respiration chambers for two to three days before the trials began. Six sessions of two days of respiratory measurements in chambers were done, performing a total of 12 days of evaluation per cow. The respiration chamber evaluation began on the 45th day after calving with a 45-day interval between sessions, for four cows at a time, as there were only four respiration chambers available. Groups of four animals went to respiration chambers; then, they were subjected to the digestibility assay in groups of eight cows; in sequence, the remaining four cows of the digestibility group went to the chambers after the digestibility. The sessions started immediately after morning feeding at 9:00 a.m. The respiratory indirect calorimetry reading was initiated, and gas exchanges were measured during 21–23 h, with an extrapolation of 24 h. The animals were randomly allocated to each chamber where they remained singly and then confined for 48 hours, leaving only for milking (morning and afternoon).

Data acquisition and analysis software (Expedata Data Analysis Software 1.8.5, version PRO, Sable Systems International) was used to calculate the consumption of O_2, CO_2, and CH_4 production (L/day). Individual enteric CH_4 production (g/day), CH_4 yield (g/kg DMI), and CH_4 intensity (g/kg milk) were calculated. Inside the chambers, there was a feeding and watering trough, and a video camera that recorded the behaviors of the animals throughout the experimental period.

Behavior within the respiration chambers

For the record of behavior, the videos (seven hours per cow, on average, performing a total of 196 h of video footages) captured by video cameras (VM 310 IR, an infrared camera from Intelbras S/A – Brazilian Electronic Telecommunications Industry, Manaus/AM, Brazil) between the two daily milking procedures at the first day of respiration chamber confinement were used. The videos of each one of the twenty eight cows were observed using focal-animal sampling and instantaneous sampling, with one-minute intervals. The following behavioral categories were used to measure cows' restlessness in the respiration chambers: lying, feeding, ruminating in the chamber, shaking ears, shaking the head, moving and being inactive, considering the time spent in each behavior, expressed in relative frequencies (%). A continuous recording was used to register the occurrences of steps, vocalization, and turning the head, expressed as number of occurrences.

Statistical analysis

First, to analyze the temperament indicators and energetic metabolism variables, a single individual measurement was obtained for each indicator, through the average of the sessions carried out throughout the study.

To assess the effects of temperament and behaviors in the chambers on the energetic metabolism and CH_4 emission measures, linear mixed models for longitudinal data were fitted by using PROC MIXED of SAS (version 9.2, SAS Institute Inc., Cary, NC). Models included the dependent variables of energetic metabolism (Fecal-E, Urine-E, CH_4-E, Heat-E, MEI/DEI, MEI/GEI, Milk-energy/MEI, NEL, EB) and CH_4 emission measures (production, yield, and intensity). Fixed effects of temperament and behavioral measures (one measure at a time), evaluation session, and their interactions, in addition to milking group, were included. The random effect of animal (subject) was considered as a repeated measure within the evaluation session. In all analyses, means were compared using posthoc Tukey Test, and P-values were assumed as significant when <0.05 and as a trend when <0.10.

For inclusion in the mixed models as fixed effects, the handling temperament, milking temperament indicators, and behavioral measures were categorized into three scores (low, average, and high). Most of the variables were classified based on the terciles of distribution (low = fist tercile, intermediate = second tercile, and high = third tercile), except by Entrance Time and Flight Distance, which were classified based on threshold values, as follows: Entrance Time ('low' = 0–9.9 s; 'intermediate' = 10–20 s; 'high' = over 20 s); Flight Distance ('low' = 0 cm; 'intermediate' = 0.1–0.9 9 cm; and 'high' = over 1 m). Finally, the behaviors such as Defecation, Rumination, KOFF that were binomial variables (occurs or not) were classified based on the number of occurrences across the 3-day session: 'low' = 0 occurrence; 'intermediate' = 1 occurrence; and 'high' = 2 or 3 occurrences. Behavioral measures in the respiration chambers (steps in the chamber, turning the head, lying, feeding, ruminating in the chamber, ear shaking, head shaking, vocation, and being inactive) were also categorized in terciles.

Results

Effects of temperament indicators on energetic metabolism and methane emissions

Regarding the effects of the milking temperament indicators, the number of STEPS showed a significant effect on Urine-E (P = 0.02), MEI/DEI (P = 0.03) and a tendency on DMI (P = 0.06) and GEI (P = 0.07) (Table 1). Similarly, a tendency for number of KICKS was found on CH_4-E (P = 0.07), CH_4 production (P = 0.09) and Heat-E (P = 0.09) (Table 1). Cows classified as intermediate for $STEPS_{Inter}$ had 26.96% lower loss of energy as urine, 2.35% higher MEI/DEI rate, and 8.98% higher gross energy intake than those classified as $STEPS_{Low}$. Either the cows defined as intermediate for $KICKS_{Inter}$ tended to show reduced losses of energy as CH_4-E, as Heat-E, and lower CH_4 production (differences of 9.19%, 7.24%, and 9.93%, respectively) than those defined as $KICKS_{Low}$ (Table 1).

The milking behaviors of rumination and kicking the milking cluster off affected NEL (P < 0.01, P = 0.04, respectively) and CH_4 intensity (P = 0.04, P = 0.03), in addition to a significant effect of rumination on Milk-energy/MEI (P < 0.01) (Table 1). Cows that kicked the milking cluster off more frequently ($KOFF_{High}$) and ruminated less frequently ($RUMINATION_{Low}$) allocated less net energy on lactation (differences of 25.24%, 57.93%, respectively) and more CH_4 intensity (36.77%, 37.10%, respectively) per liter of milk than cow classified as $KOFF_{Low}$ and $RUMINATION_{High}$, respectively. The animals classified as $RUMINATION_{High}$ had 50.00% greater Milk-energy/MEI than cows classified as $RUMINATION_{Low}$.

Concerning cows' temperament in the handling corral, Flight Speed showed a significant effect on Urine-E (P = 0.05) and a tendency on CH_4 production (P = 0.08) (Table 1). Additionally, Entrance Time affected CH_4-E (P = 0.04) and also showed a tendency on Urine-E (P = 0.08). Cows classified as Flight $Speed_{High}$ tended to lose 29.16% less energy as Urine-E and 14.29% more CH_4 production than Flight $Speed_{Low}$. Cows with Entrance $Time_{High}$ showed 35.18% more energy loss as Urine-E and 13.29% less energy loss as CH_4-E than cows with Entrance $Time_{Low}$.

Effects of behaviors in the respiration chambers on the energetic metabolism and methane emissions

The cows' behavior within the respiration chambers during the respiration assay affected some measures of energetic metabolism (Table 2). Cows that spent less time being inactive showed 2.35% less MEI/DEI (P = 0.04), and a higher frequency of vocalizations was related to 6.61% more energy loss as CH_4 (lower CH_4-E) (P = 0.03). Finally, cows that took more steps in the chamber showed a tendency of reduction of 5.65% in NEL (P = 0.10) and an increase of 12.95% in CH_4 intensity (P = 0.09) (Table 2).

Discussion

The objectives of the present study were to evaluate the effects of temperament and behavior in respiration chambers of dairy cows on energy metabolism and enteric methane emissions. Cows' temperament and behaviors in the chambers influenced energy metabolism and methane emissions, with more reactive cows allocating less energy for lactation and emitting more methane per liter of milk produced compared to calmer animals. In addition, cows with an intermediate temperament measured by steps and kicks in the milking parlor lost less energy as urine, heat and CH_4 and also produced less methane per day, compared to reactive cows.

Table 1
Effects of handling temperament and milking temperament indicators on energetic metabolism and methane emissions. Adjusted means (±SE) of energetic metabolism and methane emission measures for each temperament indicator are shown (n = 28 Holstein-Gyr dairy cows).

Item	Low	Intermediate	High	$F_{2,23}$	*P-value*
Handling Temperament Indicators					
FS (m/s)					
Urine-E (Mcal/d)	5.04 ± 0.38[a]	4.27 ± 0.27	3.57 ± 0.40[b]	3.52	0.05
CH4 Production (g/d)	229.31 ± 11.40[b]	261.43 ± 8.28[a]	262.10 ± 12.04[a]	2.88	0.08
ET (s)					
Urine-E (Mcal/d)	3.95 ± 0.32[b]	4.27 ± 0.30[b]	5.34 ± 0.49[a]	2.86	0.08
CH4-E (Mcal/d)	5.34 ± 0.14[a]	5.08 ± 0.13[a]	4.63 ± 0.22[b]	3.73	0.04
Milking Temperament Indicators					
KOFF					
NEL (Mcal/d)	12.68 ± 0.77[a]	14.37 ± 1.27[a]	9.48 ± 1.33[b]	3.67	0.04
CH4 Intensity (g/Kg milk)	19.17 ± 1.63[b]	15.49 ± 2.69[b]	26.22 ± 2.83[a]	3.92	0.03
RUMI					
NEL (Mcal/d)	9.51 ± 1.07[c]	12.41 ± 0.78[b]	15.02 ± 0.99[a]	7.19	< 0.01
Milk-energy/MEI	0.14 ± 0.01[c]	0.17 ± 0.01[b]	0.21 ± 0.01[a]	8.17	< 0.01
CH4 Intensity (g/kg milk)	25.39 ± 2.54[a]	19.07 ± 1.83[b]	15.97 ± 2.35[b]	3.83	0.04
KICKS					
CH4-E (Mcal/d)	5.33 ± 0.15[a]	4.84 ± 0.15[b]	5.30 ± 0.21[ab]	2.98	0.07
Heat-E (Mcal/d)	34.11 ± 0.83[a]	31.64 ± 0.80[b]	32.00 ± 1.16[ab]	2.65	0.09
CH4 Production (g/d)	261.54 ± 9.93[a]	235.57 ± 9.49[b]	268.68 ± 13.58[a]	2.68	0.09
STEPS					
DMI (Kg/d)	14.93 ± 0.39[b]	16.29 ± 0.41[a]	15.97 ± 0.52[ab]	3.09	0.06
GEI (Mcal/d)	66.24 ± 1.71[b]	72.19 ± 1.83[a]	70.78 ± 2.28[ab]	3.04	0.07
Urine-E (Mcal/d)	4.97 ± 0.30[a]	3.63 ± 0.32[b]	4.29 ± 0.40[ab]	4.47	0.02
MEI/DEI	0.85 ± 0.01[b]	0.87 ± 0.01[a]	0.86 ± 0.01[ab]	3.94	0.03

Abbreviations: Urine-E = % urine energy, CH_4-E = % methane energy, NEL = net energy of lactation, Milk-energy/MEI = milk-energy/ metabolizable energy intake, CH_4 intensity = methane emission, Heat-E = % heat energy, DMI = DM intake, GEI = gross energy intake, MEI/DEI = metabolizable energy intake/digestible energy intake, FS = flight speed (m/s), ET = entrance time (s), KOFF = kick off the milking cluster, RUMI = rumination, KICKS = number of kicks, STEPS = number of steps.
[a–c] Adjusted means without a common letter differ statistically from each other (Tukey test. P < 0.10).

Table 2
Effects of behaviors in the respiration chambers on the energetic metabolism and methane emissions. Adjusted means (±SE) of energetic metabolism and methane emissions measures for each behavior are shown (n = 28 Holstein-Gyr dairy cows).

Item	Low	Intermediate	High	$F_{2,50}$	P-value
Steps					
NEL (Mcal/d)	12.74 ± 0.66[a]	12.39 ± 0.68[ab]	12.02 ± 0.67[b]	2.42	0.10
CH_4 Intensity (g/kg milk)	18.37 ± 1.53[b]	20.50 ± 1.58[a]	20.75 ± 1.53[a]	2.60	0.09
Vocalization					
CH4-E (Mcal/d)	4.84 ± 0.14[b]	5.27 ± 0.12[a]	5.16 ± 0.14[a]	3.83	0.03
Inactive					
MEI/DEI	0.85 ± 0.006[b]	0.86 ± 0.005[a]	0.87 ± 0.006[a]	3.38	0.04

Abbreviations: NEL = net energy of lactation, CH_4-E = % methane energy, MEI/DEI = metabolizable energy intake/digestible energy intake.
[a–b] Adjusted means without a common letter differ statistically from each other (Tukey test. $P < 0.10$).

Effects of temperament indicators on energetic metabolism and methane emissions

Animals with temperament categorized as 'intermediate' for STEPS and KICKS lost less energy in the form of urine and had higher rates of MEI/DEI, besides presenting a tendency to produce less CH_4 and lower loss of energy as heat and CH_4. The number of leg movements has been considered a valid indicator of cows' reactivity in the milking parlor, with less reactive cows taking lower numbers of steps (Hemsworth, 2003). Nevertheless, Munksgaard et al. (2001) have observed that when some cows are kept under situations of tension and stress, they might have an opposite reaction, remaining immobile during milking. Under such perspective, it would be plausible that cows that took a few steps (as for cows in the 'intermediate' score) could be more relaxed than those that remained immobile (cows in 'low' score). Cows classified as intermediate for numbers of STEPS and KICKS showed higher DMI and could be considered more efficient as well, given the reduced losses of energy as Urine-E and CH_4-E, and lower CH_4 production. In a previous study conducted with the same animals of the present during the raising period, Ornelas et al. (2019) found a negative correlation between DMI and CH_4 production. Cows with a higher feed intake are more efficient if the metabolizable energy that exceeds maintenance is retained, associated with reduced losses of energy as urine, heat, and CH_4 (Chaokaur et al., 2014). It could explain the higher DMI in addition to lower loss of energy as urine, heat, CH_4, and higher MEI/DEI rate in cows classified as 'intermediate' for STEPS and KICKS that could be considered more efficient.

Cows that were more reactive in the milking ($KOFF_{High}$) and ruminated less in the milking parlor ($RUMINATION_{Low}$) were less efficient, allocating less net energy to milk production. Kicking the milking cluster off indicates cows' reactivity related to discomfort and emotional state of agitation (Marçal-Pedroza et al., 2020). Similarly, rumination was related to emotional states of relaxation, while its reduction could reflect tension and stress (Manteca et al., 2013). A previous study of our research group has shown that cows ruminating more frequently in the milking parlor produced 17.26% more milk than those with a lower frequency of rumination (17.59 vs. 15.00 kg/day) (Marçal-Pedroza et al., 2020). Based on the results of the present study, it is possible to infer that the increased production for more ruminating cows derives, in parts, from their better performance in allocating energy for milk production associated with lower losses as methane. This result might reveal the implications of cows' milking behaviors for the sustainability of milk production.

Cows' reactive temperament in the handling had also influenced the energy metabolism and methane emissions, with cows exiting the squeeze faster (Flight $Speed_{High}$) showed less energy in the urine and more CH_4 production, while the animals that entered faster (Entrance $Time_{Low}$) lost less energy as urine and produced more CH_4-E. It is worth to remember that the most reactive cows showed Flight $Speed_{High}$ (in m/s) and Entrance $Time_{Low}$ (in s), since they spent less time to enter into the squeeze and exit faster (high speed); thus, these measures were inversely correlated. Cows that entered and exited the squeeze chute faster (characterizing states of fear and agitation) tended to show higher losses of energy as CH_4-E and enteric CH_4 production. The flight speed and entrance time reflect an innate tendency of general fearfulness and high behavioral reactivity, revealing a susceptibility to stress in temperamental cows (the faster ones) (Cafe et al., 2011). The emotional state of fear has implications on the physiological control of metabolism, being a potential psychological stressor that leads to higher activation of the hypothalamic–pituitaryadrenal (HPA) axis, resulting in the release of glucocorticoids (Hemsworth, 2003). A relationship between reactive temperament (measured by flight speed) and susceptibility to stress was previously shown in several studies (Cafe et al., 2011). Reactive temperaments in cattle (high flight speed and crush score) were related to a more prolonged and more intense activation of HPA axis and sympatho-adrenomedullary system in responses to stress (Cafe et al., 2011). Both axes are involved in the control of catabolism, energetic homeostasis, energy balance, and storage of energy in the body. To the best of our knowledge, the present study is the first to assess the relationships between temperament, energy partitioning, and CH_4 emissions in cattle. In the study by Llonch et al. (2016), the authors investigated the relationships between beef cattle temperament (measured by flight speed and crush score), cortisol levels following transportation, and CH_4 emissions. Despite those authors not finding a relationship between flight speed and crush score with methane emissions, they reported a positive association between cortisol following transport and CH_4 emissions corrected for feed intake (g/kg DMI). Thus, the present study contributes to the scarce evidence that characteristics intrinsic to the behavior of ruminants, such as temperament, emotional states, and intensity of behavioral and physiological responses to stressors, should be taken into account in the development of alternatives to mitigate enteric CH_4 by cattle (Llonch et al., 2016, present study).

Effects of behaviors in the respiration chambers on the energetic metabolism and methane emissions

The behavior of cows in respiration chambers affected energy metabolism and methane emissions. Cows expressing behaviors indicative of restlessness (less time inactive, vocalized more and took more steps) had lower rates of MEI/DEI and lost more energy as CH_4, and tended to allocate less NEL and more CH_4 intensity. For confined beef cattle, Llonch et al. (2018) showed that a higher level of activity in the home pens (measured as number of steps per day) was related to lower feed efficiency (poorer residual feed intake), which the authors attributed to the higher energy expenditure for muscle activity in more active individuals. Additionally, in beef cattle, efficient animals show lower maintenance requirements as well as better usage of metabolizable energy for growth

(Cantalapiedra-Hijar et al., 2018). These results might explain the lower MEI/DEI and lower NEL in cows that took more steps, which probably were less efficient.

Vocalizations and steps in situations involving physical restraint can be used as indicators of cows' restlessness since confinement and social isolation are stressors for social animals (Llonch et al., 2018). Restless cows might lose more energy as CH_4-E, allocating less energy for milk yield, in parts, due to more intense physiological responses to stress in these animals. Stress responses are detrimental for efficiency in energy use, leading to reduced productivity and the rise of enteric CH_4 emissions (Hedlund and Løvlie, 2015; Llonch et al., 2018). On the other hand, calmer and relaxed cows might have the potential to be more productive and efficient in energy partitioning and use, along with CH_4 intensity reduction per unity of product (Yan et al., 2010).

Our study has some limitations that have to be taken into account. First, the measures of metabolism and methane emissions were taken in potentially stressful situations. Both tie-stall and respiration chambers involve physical restraint and reduced social interactions, in spite of the visual contacts were maintained. All the cows were exposed to the same experimental conditions when they were heifers (Ornelas et al., 2019). The heifers went through ten days of adaptation to the tie-stall and four days of adaptation in the respiration chambers, followed by a 5-day digestibility assay and two days in the respiration chambers. The feed intake was monitored by collecting and weighing feed leftovers to ensure they did not exceed 10%, as a measure of behavioral changes in tie-stall and chambers. Thus, we expect that all the cows were adapted to this study's conditions, leading us to consider our results valid; even so, caution is required when extrapolating our findings to non-experimental or commercial conditions. A second limitation was the lack of ruminal microbiome community assessment in our study. It is known that the ruminal microbiome composition plays an important role in cows' feed efficiency, energy utilization, and methane emissions (Difford et al., 2018; Schären et al., 2018) and should have affected our results.

In summary, reactive temperament, stress, and welfare problems potentially cause additional energy expenditure for animals to cope with such situations. Beyond the economic losses caused by the inefficient use of feeding resources and reduced milk yield, the reactive temperaments of cattle might cause concerns related to the risks of accidents and deteriorate the labor conditions in dairy farms (Hemsworth, 2003; Sutherland and Huddart, 2012). Finally, this study has shown that environmental consequences might arise from the increasing CH_4 emissions for temperamental cattle. All these factors are integrated within the perspective of 'One Welfare' (García et al., 2016; Tarazona et al., 2019). Thus, we recommend the improvement of temperament throughout animal breeding and good practices of cattle handling as viable strategies for attaining a more sustainable dairy production.

Conclusion

Cattle temperament assessed during milking and in the handling corral, in addition to cows' behaviors within the respiration chambers, were related to energy partitioning and CH_4 emissions by crossbred dairy cows under the experimental conditions of the present study. Animals classified as more reactive allocated less energy for lactation and emitted more enteric CH_4 per unity of product. All those impacts of reactive temperaments are undesirable for an efficient and sustainable livestock activity. A selection of calmer cows and the adoption of good practices of cattle handling could favor the welfare of cows, stockpeople, and the environment.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100224.

Ethics approval

This research was approved by the Embrapa Dairy Cattle Animal Care and Use Committee, Juiz de Fora, MG, Brazil (Protocol 5201240417), being conducted according to the ethical principles of animal experimentation.

Data and model availability statement

None of the data was deposited in an official repository. Data and models used may be available upon request by contacting the corresponding author.

Author ORCIDs

M.G. Marçal-Pedroza: https://orcid.org/0000-0002-0390-3390.
M.M. Campos: https://orcid.org/0000-0001-5332-6010.
J.P. Sacramento: https://orcid.org/0000-0001-8451-7338.
L.G.R. Pereira: https://orcid.org/0000-0001-7166-5817.
F.S. Machado: https://orcid.org/0000-0002-7380-4961.
M.J.R. Paranhos da Costa: https://orcid.org/0000-0002-0088-4008.
A.C. Sant'Anna: https://orcid.org/0000-0002-5431-7199.

Author contributions

M.G. Marçal-Pedroza: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Project administration.

M.M. Campos: Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

J.P. Sacramento: Methodology, Investigation, Data curation, Writing - review & editing.

L.G.R. Pereira: Conceptualization, Methodology, Resources, Writing - Review & editing, Supervision, Funding acquisition.

F.S. Machado: Methodology, Resources, Writing - review & editing, Funding acquisition.

T.R. Tomich: Methodology, Resources, Writing - review & editing, Funding acquisition.

M.J.R. Paranhos da Costa: Methodology, Writing - review & editing, Funding acquisition.

A.C. Sant'Anna: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of Interest

The authors declare no conflict of interest.

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