Characterizing the metabotype and its persistency in lactating Holstein cows: An approach toward metabolic efficiency measures

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ABSTRACT

The variation in feed efficiency among dairy cows is due to differences in fermentation and digestion characteristics, but recent studies have suggested that various aspects of postabsorptive metabolic processes including heat production or the metabolizable energy for maintenance are more crucial. Thus, metabolic efficiency largely determines feed efficiency, but whether divergent feed efficient cows differ in O₂ consumption and metabolic CO₂ production, directly determining the metabolic rate has not been investigated. Therefore, the objective of the present study was to determine whether variation in ME intake (MEI), O₂ consumption, and metabolic CO₂ production account for the variation in metabolic efficiency of dairy cows and whether this effect persists across the lactation cycle. Eighteen cows with different German breeding value functional herd life were kept in freestalls with ad libitum access to a total mixed ration that was kept constant in composition throughout the first lactation. Cows were blood sampled and weighed at wk 5, 13, and 42 postpartum (pp) and transferred into respiration chambers. Animals were retrospectively clustered according to MEI, O₂ consumption, and metabolic CO₂ production, each normalized to metabolic body weight (mBW). Cluster analysis revealed 9 high metabolically efficient (high-Meff) and 9 low metabolically efficient cows. The high-Meff cows had greater MEI and feed conversion efficiency, produced less metabolic CO₂ and methane, had a stronger negative energy balance, and tended to have a lower metabolic respiratory quotient. Further, high-Meff cows had lower residual MEI, less heat energy loss, and lower plasma glucose concentrations, but used a greater portion of body reserves instead of feed energy for milk synthesis, particularly at wk 5 and 13 pp. However, these group differences did not persist by wk 42 pp. Cow groups were not different

in O₂ consumption, milk yield, metabolizable energy for maintenance, or the efficiency of tissue utilization for milk synthesis, but high-Meff cows tended to have the lower German relative breeding value functional herd life, indicating a link between metabolic performance and productive lifespan. In conclusion, the use of a clustering approach involving MEI/mBW, O₂/mBW, and CO₂/mBW seems to be a promising method to differentiate cows with divergent metabolic efficiency but does not allow identifying an individual metabotype that persists across the whole lactation cycle.

Key words: energy metabolism, metabolic efficiency, lactation, dairy cow

INTRODUCTION

The global demand for meat and dairy products continues to increase primarily because of an expanding world human population and increase in lifespan. On the other hand, the size of global farm land for the production of feed for animals decreases steadily (Salter, 2017) and feed costs amount to the largest proportion of overall costs at least in pasture-based systems of milk production (Hanrahan et al., 2018). Therefore, improving feed efficiency of dairy cows is still an urgent need, although it has increased substantially in the past decades (Capper et al., 2009).

Feed efficiency, in general, is the proportion of feed converted to products such as milk and meat. For dairy cows, feed conversion efficiency (**FCE**) is defined as the ratio of kilograms of milk per kilogram of DMI. Another measure of feed efficiency is residual feed intake (**RFI**), which was first defined as the difference between actual and predicted feed intake of beef cattle (Koch et al., 1963). Five major biological processes involving feed intake pattern, fermentation and digestion of feed, anabolic and catabolic postabsorptive metabolism, physical activity, and thermoregulation determine the level of feed efficiency of a healthy cow (Herd and Arthur, 2009). Partial contributions of each of these biological processes may vary between animals, but none of these processes has been included in the definition of FCE or

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RFI. However, some recent studies investigated different components of feed efficiency in divergent RFI cows. For example, dairy cows classified as being highly feed efficient (low RFI) differ in their rumen microbial community composition compared with less feed efficient (high RFI) counterparts (Jewell et al., 2015). Also, low RFI cows were characterized by a greater apparent N digestibility, by a trend for greater digestibility of DM and of OM, as well as by lesser excretion of feed N as fecal N (Rius et al., 2012). The greater digestibility in low RFI cows may not only depend on a more effective microbiome and digestive ability but also on diet composition. In a recent study it was demonstrated that digestive ability was not different between low and high RFI cows on a high starch diet but varied between 9 to 31% between animals on low starch diets (Potts et al., 2017). These authors speculated that differences in postabsorptive metabolic processes, heat production, or energy utilization for maintenance account more for the variation in RFI than digestibility (Potts et al., 2017). Approaches to directly measure metabolic heat production and energy utilization for maintenance require experiments in metabolic chambers. Only a few studies have been conducted on the comparison of maintenance energy requirements or heat production of mid- and late-lactating dairy cows with contrasting feed efficiencies (Arndt et al., 2015; Dong et al., 2015). High-FCE cows partitioned 15% more feed energy toward milk but also maintenance functions, secreted less portion of feed N through urine, and produced less heat than low FCE counterparts (Arndt et al., 2015). Thus, high FCE cows possess a greater metabolic efficiency (conversion of postabsorptive feed energy to milk) both during mid and late lactation (Arndt et al., 2015), suggesting that a persisting metabolotype of an animal determines its feed efficiency to a large extent.

Within the first 5 wk of early lactation, dairy cows may use 46 kg of body fat and 12 kg of body protein for milk synthesis and maintenance (Komaragiri et al., 1998). Therefore, good estimates for feed efficiency of dairy cows need to account, besides feed energy intake and milk energy losses, also for body mass changes, stage of lactation, and parity (Hurley et al., 2016; Potts et al., 2017). Arndt et al. (2015), however, did not investigate whether the differences in the cow's feed efficiency and metabolotype already existed in early lactation and whether the portion of tissue energy used for milk production differed between divergent FCE cows.

Feed efficient (low-RFI) beef cattle were characterized to have lower methane (CH_4) losses and lower metabolic rates; the latter is the amount of ME expended as heat per kilogram of metabolic body weight, as compared with less feed efficient counterparts (Nkrumah et al., 2006). The metabolic rate is directly related to the

amount of O_2 consumed and CO_2 produced, and the level of total splanchnic tissue O_2 consumption tended to be higher in steers with high-FCE (higher ADG per kg of DMI; Hersom et al., 2003). However, whether divergent FCE cows differ in O_2 consumption and metabolic CO_2 production in dependency of energy intake has not been investigated. Therefore, the objective of the present study was to determine whether variation in ME intake (**MEI**), O_2 consumption, and metabolic CO_2 production accounts for the variation in metabolic efficiency, and if so whether this effect persists through the course of lactation.

MATERIALS AND METHODS

Animals and Experimental Design

The experimental protocol was approved by the local animal ethics committee (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern; approval no. 7221.1–1.-053/13). Eighteen German Holstein cows at first calving between October 2013 and January 2014 were used from the herd at the Leibniz-Institute for Farm Animal Biology, Dummerstorf, Germany. The herd included 18 animals with a high German relative breeding value functional herd life (mean $\pm$ SE: 121 ± 1 ; range from 109–128) and 18 animals with a low German relative breeding value functional herd life (96 ± 1 , range from 85–106). Breeding values were determined from genomic data (Masterrind, 2019). Animals used for the present study were randomly selected from the 2 genotype groups. Cows were kept in freestalls with ad libitum access to water and fed a TMR that was kept constant in composition throughout the first lactation. Diet composition was formulated according to recommendations of the German Society of Nutrition Physiology (GfE, 2004). Before calving, animals were halter-trained and well adapted to respiration chambers at least 3 times until the animal showed regular eating, drinking, ruminating behavior, and lying down.

Sample Collection and Respiration Chamber Measurements

At wk 5, 13, and 42 (SE $\pm$ 0.2) postpartum (**pp**), cows were blood sampled directly before transfer to respiration chambers. Blood samples were collected from the left jugular vein in 9-mL monovettes containing EDTA (Sarstedt, Nümbrecht, Germany). Monovettes were immediately placed on ice and centrifuged at $1,570 \times g$ for 20 min at 4°C to obtain plasma which was stored at -80°C before analysis. Plasma nonesterified fatty acids (**NEFA**), triglycerides, BHB, and glucose concentra-

tions were analyzed photometrically (Abx Pentra 400, Horiba, Kyoto, Japan) using the following kits: NEFA-HR(2) (Wako Chemicals, Neuss, Germany) for NEFA, #A11A01640 (Abx-Horiba) for triglycerides, #RB 1008 (Labor+Technik Lehmann, Berlin, Germany) for BHB, and #A11A01667 (Axon Lab, Reichenbach, Germany) for glucose.

Cows were weighed to measure BW directly before and after respiration chamber measurement to calculate mean metabolic body weight (**mBW**):

$$\text{mBW} = \text{BW}^{0.75}. \quad [1]$$

Within chambers, animals were kept in tiestall at 15°C, with a dark-light cycle from 0600 to 1900 h and ad libitum access to water. A TMR sample was taken at each time of respiration chamber measurements for the analysis of DM and diet composition. Feed analyses were conducted by the Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA, Rostock, Germany). The chemical composition of the TMR is shown in Table 1. Based on the results obtained for CP, crude fiber (CFi), and crude fat (CFa), ME content of the diet was calculated according to Boguhn et al. (2003):

$$\begin{aligned} \text{ME (MJ/kg of DM)} &= 6.0756 + 0.19123\text{CFa} \\ &+ 0.02459\text{CP} - 0.000038\text{CFi}^2 - 0.002139\text{CFa}^2 \\ &- 0.000060\text{CP}^2. \end{aligned} \quad [2]$$

After an overnight stay allowing gas exchange equilibration, measurement of gas concentrations started at 0700 h. On d 1 in the chamber, animals were fed at 0700 and 1630 h for ad libitum intake. The amount of feed consumed was determined every 6 min by feed disappearance from the trough placed on an electronic scale for 24 h. From this, daily DMI and MEI were calculated. The residual metabolizable energy intake (**RMEI**) was calculated as the difference between the actual MEI and the theoretical MEI (MEI_{theo}) calculated from the BW, milk yield, and milk constituents using the software HERDEplus (dsp agrosoft GmbH, Paretz, Germany), which is based on the GfE (2004) equations.

At 0700 h on d 2, feed residuals were removed and gas exchanges were measured for further 24 h (fasting period). The airflow through the chamber amounted to approximately 30 m³ per h and was measured by a differential-pressure type V cone flow meter (McCrometer, Hemet, CA). Concentrations of CO₂ and CH₄ in the chamber were analyzed by infrared-absorption and the concentration of O₂ was analyzed paramagnetically (SIDOR SICK AG, Waldkirch, Germany) every 6 min.

As total CO₂ production (V_{CO_2}) is the sum of fermentative (CO₂ _{ferm}) and metabolic CO₂ (CO₂ _{metab}), CO₂ _{ferm} was estimated according to Chwalibog et al. (1996):

$$V_{\text{CO}_2 \text{ ferm}} (\text{L}) = 1.7 \times V_{\text{CH}_4} (\text{L}), \quad [3]$$

in which the factor 1.7 is constant for a variety of diet compositions. Accordingly, $V_{\text{CO}_2 \text{ metab}}$ was calculated by subtracting $V_{\text{CO}_2 \text{ ferm}}$ from V_{CO_2} and the metabolic respiratory quotient (**RQ**_{metab}) as $V_{\text{CO}_2 \text{ metab}}/V_{\text{O}_2}$ (Derno et al., 2013). Energy expenditure (**EE**) was calculated according to the following equation (Brouwer, 1965):

$$\begin{aligned} \text{EE (kJ)} &= 16.18V_{\text{O}_2} (\text{L}) + 5.02V_{\text{CO}_2} (\text{L}) \\ &- 2.17V_{\text{CH}_4} (\text{L}) - 5.99N_{\text{u}} (\text{g}), \end{aligned} \quad [4]$$

where N_{u} is urine N excretion. Physical-activity-related EE (**EE**_{act}) was calculated as the difference between EE and the mean of the 10 lowest EE values over the day, which are an estimate of resting EE. The mean EE measured during the last 30 min of the feed restriction period on d 2 (**EE**_{rest}) and **EE**_{act} was used to calculate diet-induced thermogenesis (**DIT**) according to

Table 1. Ingredients and nutrient composition of diet

Diet composition	Concentration
Ingredient	
Corn silage, g/kg of DM	325
Grass silage, g/kg of DM	181
Hay, g/kg of DM	32
Straw, g/kg of DM	38
Corn kernels, g/kg of DM	70
Concentrate MF2000, ¹ g/kg of DM	220
Grain mix, g/kg of DM	39
Extracted soy meal, g/kg of DM	21
Extracted canola meal, g/kg of DM	38
Molassed sugar beet pulp, g/kg of DM	23
Mineral/vitamin mix, ² g/kg of DM	9
Lime, g/kg of DM	4
Nutrient composition	
Crude fat, g/kg of DM	31
CP, g/kg of DM	152
Crude fiber, g/kg of DM	168
Starch, g/kg of DM	247
Ash, g/kg of DM	69
Energy content	
GE, MJ/kg of DM	17.0
ME, MJ/kg of DM	11.2

¹33% extracted soy meal, 20% corn, 17% wheat gluten, 13% wheat, 8% extracted rapeseed meal, 5% sugar beet pulp, 2% sodium hydrogen carbonate, 1.3% calcium carbonate, and 0.2% sodium chloride (Vollkraft Mischfutterwerke GmbH, Güstrow, Germany).

²Rinderstolz 9522 Salvana (Tierernährung, Kl.-O. Sparrieshoop, Germany; per kg of feed; 88% DM): 39.3% calcium carbonate, 21.7% monocalcium phosphate, 21% sodium chloride, 11.9% magnesium oxide, and 2% sugar beet molasses. Composition: 92% crude ash, 20% calcium, 8% sodium, 6% magnesium, 5% phosphorus, 1,000,000 IU of vitamin A, 200,000 IU of vitamin D₃, and 4,500 mg of vitamin E. No information on trace minerals was provided by the supplier.

$$\text{DIT (kJ)} = \text{EE} - \text{EE}_{\text{rest}} - \text{EE}_{\text{act}}. \quad [5]$$

Loss of body tissue energy or energy balance (**EB**) for each stage of lactation during ad libitum feeding was calculated according to Kirkland et al. (2002):

$$\text{EB (kJ)} = \text{MEI (kJ)} - \text{EE (kJ)} - \text{ECM (kJ)}. \quad [6]$$

Urine N excretion (N_u) was not measured but estimated to 50 g/d, although real N_u may amount to 75 to 150 g/d (Dijkstra et al., 2013), thereby accepting an error of about 0.4% in the absolute EE values. Animals were milked twice daily (0630 and 1630 h). Milk yield was recorded and samples from the evening and subsequent morning milking were pooled and sent to the Milk Testing Laboratory (Güstrow, Germany) for the analyses of milk fat, protein, and lactose using infrared spectrophotometry (MilkoScan, Foss GmbH, Rellingen, Germany) to calculate ECM as follows:

$$\begin{aligned} \text{ECM (kg)} = & [38 \times \text{fat (g)} + 24 \times \text{protein (g)} \\ & + 17 \times \text{lactose (g)}] \times \text{milk (kg)} / 3,140, \end{aligned} \quad [7]$$

$$\begin{aligned} \text{ECMe (kJ)} = & [38 \times \text{fat (g)} + 24 \times \text{protein (g)} \\ & + 17 \times \text{lactose (g)}] \times \text{milk (kg)}. \end{aligned} \quad [8]$$

To assess the relationship between MEI, milk energy (**ECMe**), and EB, multiple regression analysis was performed on a per unit mBW basis [$\text{kJ}/(\text{kg}^{0.75}\text{d})$] according to Kirkland et al. (2002):

$$\begin{aligned} \text{ECMe/mBW} = & a + b (\text{MEI/mBW}) \\ & + c (\text{EB/mBW}) [\text{kJ}/(\text{kg}^{0.75}\text{d})], \end{aligned} \quad [9]$$

where b provides an estimate of the efficiency of ME used for ECM production (k_l), and if EB is negative, the coefficient c is an estimate of the efficiency of tissue energy mobilization for ECM production (k_{lt}).

The quotient $-a/b$ is an estimate of maintenance energy requirement (**ME_m**):

$$\text{ME}_m/\text{mBW} = -a/b [\text{kJ}/(\text{kg}^{0.75}\text{d})]. \quad [10]$$

The coefficients determined in equation [9] and ME_m obtained from equation [10] were used to calculate the partitioning of milk production from intake and tissue mobilization, respectively:

$$\begin{aligned} \text{MEIM/mBW} = & \text{MEI/mBW} \\ & - \text{ME}_m/\text{mBW} [\text{kJ}/(\text{kg}^{0.75}\text{d})], \end{aligned} \quad [11]$$

in which MEIM is the portion of MEI used for milk production.

$$\begin{aligned} \text{ECMe}_{\text{int}}/\text{mBW} = & \text{MEIM/mBW} \\ & \times k_l [\text{kJ}/(\text{kg}^{0.75}\text{d})], \end{aligned} \quad [12]$$

$$\begin{aligned} \text{ECMe}_{\text{tiss}}/\text{mBW} = & \text{ECMe/mBW} \\ & - \text{ECMe}_{\text{int}}/\text{mBW} [\text{kJ}/(\text{kg}^{0.75}\text{d})], \end{aligned} \quad [13]$$

in which ECMe_{int} and $\text{ECMe}_{\text{tiss}}$ are the milk energy produced from feed intake and tissue mobilization, respectively.

$$\begin{aligned} \text{EEM}_{\text{int}}/\text{mBW} = & \text{MEIM/mBW} \\ & \times (1 - k_l) [\text{kJ}/(\text{kg}^{0.75}\text{d})], \end{aligned} \quad [14]$$

$$\begin{aligned} \text{EEM}_{\text{tiss}}/\text{mBW} = & \text{EB/mBW} \\ & \times (1 - k_{lt}) [\text{kJ}/(\text{kg}^{0.75}\text{d})], \end{aligned} \quad [15]$$

in which EEM_{int} and EEM_{tiss} are the energy expenditure of milk production from feed intake and tissue mobilization, respectively.

$$\text{ECM}_{\text{int}} = \text{ECMe}_{\text{int}}/3,140 \text{ (kg)} \quad [16]$$

$$\text{ECM}_{\text{tiss}} = \text{ECMe}_{\text{tiss}}/3,140 \text{ (kg)} \quad [17]$$

where ECM_{int} and ECM_{tiss} are the energy-corrected milk yield produced from feed intake and tissue mobilization, respectively.

In addition and alternatively to the use of equation [9], k_{lt} was also calculated by

$$k_{lt} = \text{ECM}_{\text{tiss}}/(-\text{EB}) \quad [18]$$

for stages in negative EB.

Statistical Analyses

To identify individual animals of similar metabotype, hierarchical cluster analysis was performed using the Ward method in JMP (version 12.0.1, SAS Institute Inc., Cary, NC). Animals were clustered using the 3 metabotype characterizing variables MEI/mBW, $V_{\text{CO}_2 \text{ metab}}/\text{mBW}$, and $V_{\text{O}_2}/\text{mBW}$ as measured at wk 5 pp during ad libitum feeding, because it was assumed that the greatest metabolic differences occurred in early lactation. Based on the obtained dendrogram, cows clustered into 3 groups consisting of 9, 7, and 2 animals (Figure 1). Because of the similarity of 2 of the clusters, groups with the 7 and 2 animals were unified to one group, yielding 2 groups of each 9 cows. Animals clus-

Figure 1. Hierarchical clustering of 18 cows according to metabolizable energy intake (MEI), metabolic CO₂ production, and O₂ consumption per kilogram of metabolic body weight (mBW) at wk 5 postpartum. The numbers on the y-axis refer to the animal number, and the order reflects their rank. Colors code for MEI/mBW, V_{CO₂ metab}/mBW, and V_{O₂}/mBW values, respectively, with dark red illustrating the highest and light yellow the lowest value in each column. V_{CO₂ metab} = volume of produced metabolic CO₂; V_{O₂} = volume of O₂ consumed. Low Meff = low metabolic efficiency; high Meff = high metabolic efficiency.

tering to high MEI/mBW, V_{CO₂ metab}/mBW, and V_{O₂}/mBW values were considered as being low metabolic efficient (**low-Meff**), whereas those with low MEI/mBW, V_{CO₂ metab}/mBW, and V_{O₂}/mBW were classified as being high metabolic efficient (**high-Meff**).

Statistical analysis was performed using a 2-way repeated measures ANOVA model with the fixed factor group (high-Meff and low-Meff), the repeated factor time (wk 5, 13, and 42), and the interaction of group × time using the MIXED procedure of SAS (SAS Systems, version 9.4, SAS Institute Inc., Cary, NC). To evaluate the differences between the 2 groups at each time, the SLICE option of PROC MIXED was used. The covariance was modeled as unstructured. All statistical tests were performed for a significance level of $P < 0.05$. A trend was considered at $P < 0.1$.

RESULTS

Contrary to the clustering criteria, V_{O₂}/mBW did not differ between cow groups, but in accordance with the

presetting, high-Meff cows had lower MEI/mBW and V_{CO₂ metab}/mBW compared with low-Meff cows at wk 5 pp ($P < 0.05$; Table 2). However, these group differences did not exist at wk 13 and 42 pp. The difference in MEI/mBW at wk 5 after calving was not due to differences in BW, but MEI and DMI, respectively, were lower in high-Meff compared with low-Meff cows ($P < 0.05$). High-Meff cows produced less metabolic CO₂ ($P < 0.05$) and tended to have a lower RQ_{metab} value ($P = 0.06$) at wk 5 pp, whereas these differences were not apparent at wk 13 and 42 pp. Both cow groups did not differ in daily O₂ consumption, but CH₄ production, CH₄/mBW, and CH₄/ECM were lower in high-Meff relative to low-Meff counterparts at wk 5 and 13 pp ($P < 0.05$), whereas CH₄ yield (CH₄/DMI) did not differ between groups. In addition, the number of DIM when measured in chambers, milk constituents, and ECM were not different between cow groups; however, FCE was higher in high-Meff than low-Meff cows at wk 5 and 13 pp ($P < 0.05$). Vice versa, RMEI and the EB was always lower in high-Meff compared with low-Meff

cows ($P < 0.05$). Diet-induced thermogenesis DIT/mBW and heat production expressed as EE/mBW were lower in high-Meff cows at wk 5 but not wk 13 and 42 pp ($P < 0.05$). No differences were observed in heat production during ad libitum feeding (EE) or after the 24 h-feed restriction period (EE_{rest}/mBW) between groups, but BW loss after the 24 h-feed restriction period increased from wk 5 to wk 42 pp. Interestingly, loss of BW after feed restriction was significantly higher in high-Meff cows ($P_{\text{group}} < 0.05$). With the exception of total O₂ consumption, all variables tended to change ($P_{\text{time}} < 0.1$) or changed ($P_{\text{time}} < 0.05$) from wk 5 to 42 pp without significant group $\times$ time interactions.

As each of the divergent efficiency groups consisted of only 9 cows, multiple-regression analysis was performed for each time point including all 18 cows. As a result, the constant *a* ranged between -516.2 and -446.1 , coefficient *b* between 0.65 and 0.69 , and coefficient *c* between -0.73 and -0.84 (Table 3). Each of the 3 regression models had an adjusted R² value of 0.95 . By using the 3 regression models, daily ME_m/mBW was calculated to amount to 743 , 686 , and 738 kJ/kg^{0.75} for wk 5, 13, and 42 pp, respectively. With these values, the MEIM was calculated for each group and found to be lower in high-Meff cows at wk 5 pp ($P < 0.05$). Moreover, the comparison between the absolute value of coefficient *c* obtained from the regression model (equation [9]) and the *k_{lt}* value calculated by equation [18] revealed good agreement of both estimates.

Next, the regression models were used to calculate several measures of metabolic efficiency of milk production for each of the 2 groups. The portion of milk produced from feed intake (ECM_{int}) as well as the portion of milk energy produced per unit mBW from feed intake (ECME_{int}/mBW) were lower in high-Meff than low-Meff cows at wk 5 pp ($P < 0.05$) and peaked at wk 13 for both groups ($P_{\text{time}} < 0.05$). On the other hand, the portion of milk produced from endogenous tissue sources (ECM_{tiss}) and the milk energy produced per mBW from tissue (ECME_{tiss}/mBW) were greater in high-Meff than low-Meff cows at wk 5 and 13 ($P < 0.05$) and declined in both groups by wk 42 pp ($P_{\text{time}} < 0.05$). The ECME_{int}/ECME_{tiss} ratio was even lower than 1 in high-Meff cows 5 wk after calving and increased with progressing time after calving in both groups ($P_{\text{time}} < 0.05$). The energy expended for milk production from feed intake (EEM_{int}/mBW) was significantly lower in high-Meff than low-Meff cows at wk 5 pp ($P < 0.05$), whereas no differences were observed at later time points. The energy expended for milk production from mobilized tissue (EEM_{tiss}/mBW) was not different between groups at any of the 3 stages, but there was an overall group effect with higher values in high-Meff cows ($P_{\text{group}} < 0.05$). To verify this result,

we calculated EEM_{tiss}/mBW (equation [15]) using the *k_{lt}* value determined from the regression equation [9] and calculated from equation [18]. The results based on the *k_{lt}* value obtained from equation [18] revealed that EEM_{tiss}/mBW was higher ($P < 0.05$) or tended to be higher ($P = 0.08$) for high-Meff cows at all time points after calving. In addition, the accuracy of the regression model was examined by calculating the sum of ME_m + EEM_{int} + EEM_{tiss} (Table 3), where the summands were obtained from the equations [10], [14], and [15]. The sum calculated equals to EE/mBW and is almost identical to the value measured by indirect calorimetry with lower numbers of high-Meff cows at wk 5 pp.

Plasma NEFA, concentrations as markers for adipose tissue mobilization, respectively, were not different between groups, but plasma glucose concentrations tended to be ($P = 0.06$) or were lower in high-Meff cows at wk 5 and 13 pp ($P < 0.05$; Table 4). Plasma triglyceride concentrations as an indicator of fat transfer into milk and BHB concentrations reflecting ketosis were also not different between cow groups. In addition, low-Meff cows tended to have a higher relative German breeding value functional herd life as compared with high-Meff cows (118 ± 4 vs. 107 ± 4 ; $P = 0.1$), whereas the German relative breeding value milk production (Masterrind, 2019) did not differ between groups (117 ± 2 vs. 117 ± 4 ; $P = 0.96$).

DISCUSSION

Clustering Results and Maintenance Energy Requirement

The objectives of the study were to assess whether variables of energy expenditure account for the variation in metabolic efficiency of dairy cows and whether differences in these variables persist at various stages after calving. To approach this aim, we clustered 18 cows according to the 3 measured variables MEI, O₂ consumption, and metabolic CO₂ production, each normalized to the mBW, because a complex trait such as metabolic efficiency is difficult to define by a sole variable only. We found that the 18 cows could be separated in 2 groups with high-Meff cows exhibiting less MEI/mBW and V_{CO₂ metab}/mBW than low-Meff cows despite of their comparable BW and ECM production. However, O₂ consumption as another clustering criterion was not different between these groups, which seem to contrast earlier reported differences in splanchnic tissue O₂ consumption per mBW between high and low FCE steers (Hersom et al., 2003). On the other hand, O₂ consumption alone was assessed to be an insufficient marker of energy metabolism, as rather the amount of ATP generated per oxygen atom (P/O ratio) by the

Table 2. Least squares means and pooled standard error (PSE) of BW, intake, milk, and gas exchange variables of dairy cows with low (low-Meff, n = 9) and high (high-Meff, n = 9) metabolic efficiency at wk 5, 13, and 42 postpartum

Item ¹	Wk 5				Wk 13				Wk 42				P-value ²
	Low-Meff	High-Meff	PSE	P-value ³	Low-Meff	High-Meff	PSE	P-value ³	Low-Meff	High-Meff	PSE	P-value ³	
BW, kg	566	560	17	0.79	566	551	17	0.54	632	638	22	0.84	
BW loss, kg	65	75	4	0.13	73	87	4	0.01	69	93	6	0.01	<0.01
DMI, kg/d	14.5	11.2	0.48	<0.01	15.0	13.8	0.64	0.22	15.9	14.3	0.72	0.15	<0.01
MEI, MJ/d	163.8	126.0	5.72	<0.01	166.6	154.3	7.03	0.24	179.7	162.1	8.01	0.14	<0.01
MEI/mBW, MJ/(kg ^{0.75} d)	1.41	1.09	0.04	<0.01	1.46	1.36	0.07	0.33	1.44	1.29	0.08	0.21	<0.05
V _{CO2} metab, L/d	4,823	4,382	116	<0.05	5,052	4,952	112	0.54	5,451	5,276	150	0.42	<0.01
V _{CO2} metab/mBW, L/(kg ^{0.75} d)	41.6	38.0	0.64	<0.01	44.2	43.7	1.3	0.77	43.4	41.8	1.5	0.46	<0.01
V _{O2} , L/d	5,721	5,424	163	0.22	5,797	5,704	125	0.61	5,976	5,887	135	0.65	0.27
V _{O2} /mBW, L/(kg ^{0.75} d)	49.4	47.1	1.0	0.14	50.7	50.2	1.1	0.77	47.6	46.6	1.5	0.64	<0.01
RQ _{metab}	0.84	0.81	0.01	0.06	0.87	0.87	0.02	0.90	0.91	0.89	0.01	0.15	<0.01
CH ₄ , L/d	446	353	20	<0.01	482	430	17	<0.05	531	489	25	0.31	0.01
CH ₄ /mBW, L/(kg ^{0.75} d)	3.85	3.07	0.15	<0.01	4.24	3.78	0.13	<0.05	4.21	3.96	0.19	0.37	<0.01
CH ₄ /DMI, L/kg	30.9	31.9	1.5	0.64	32.4	31.5	1.3	0.63	33.7	35.2	1.7	0.53	0.07
CH ₄ /ECM, L/kg	14.4	11.3	1.0	<0.05	17.9	15.1	0.8	<0.05	24.2	21.6	1.3	0.17	<0.05
Pregnancy, d	—	—	—	—	—	—	—	—	99	127	25	0.45	—
DMI, d	28	27	2.2	0.92	88	86	1.5	0.49	293	290	2.5	0.98	—
Milk, kg/d	29.1	29.3	1.7	0.91	27.4	28.6	1.43	0.55	20.2	22.5	1.50	0.27	<0.01
Milk fat, %	4.79	5.07	0.39	0.69	3.94	4.07	0.18	0.63	4.37	4.53	0.28	0.68	<0.01
Milk protein, %	3.09	3.07	0.08	0.87	3.25	3.20	0.06	0.57	3.83	3.85	0.02	0.93	<0.01
Milk lactose, %	4.96	4.85	0.06	0.22	4.98	5.00	0.06	0.76	4.88	4.88	0.06	0.99	<0.01
ECM, kg/kg	31.7	32.9	2.21	0.70	27.5	28.7	1.34	0.52	21.9	22.1	2.28	0.95	<0.01
FCE, kg/kg	2.21	2.98	0.16	<0.01	1.83	2.10	0.07	0.01	1.39	1.49	0.15	0.63	<0.01
RMEI, MJ/d	—63.2	—107.8	10.4	<0.01	—37.9	—55.9	4.7	0.01	0.72	—26.4	8.3	0.03	<0.01
MEI _{theo} , MJ/d	227	234	12	0.68	204	210	7	0.56	179	193	7	0.15	<0.01
ECMe/mBW, kJ/(kg ^{0.75} d)	863	899	59	0.67	758	795	41	0.53	549	560	54	0.89	<0.01
EB, MJ/d	—55.0	—89.0	7.5	<0.01	—40.5	—55.5	4.4	<0.05	—16.3	—32.0	6.3	0.09	<0.01
EE, MJ/d	119	111	3	0.11	121	119	2	0.52	127	124	3	0.54	<0.05
EE _{rest} /mBW, kJ/(kg ^{0.75} d)	710	701	14	0.65	697	724	16	0.25	622	639	12	0.34	<0.01
EE _{act} /mBW, kJ/(kg ^{0.75} d)	154	152	7	0.87	158	172	11	0.38	153	142	9	0.41	<0.05
DIT/mBW, kJ/(kg ^{0.75} d)	167	118	15	0.03	201	157	22	0.17	118	207	19	0.24	<0.05

¹BW loss = difference between ad libitum feeding and 24-h feed restriction; MEI = metabolizable energy intake; mBW = metabolic body weight; V_{CO2} metab = volume of produced metabolic CO₂; V_{O2} = volume of O₂ consumed; RQ_{metab} = metabolic respiratory quotient; FCE = feed conversion ratio; RMEI = residual metabolizable energy intake; MEI_{theo} = theoretical MEI calculated from the BW, milk yield, and milk constituents; ECMe = milk energy; EB = energy balance; EE = energy expenditure; EE_{rest} = energy expenditure during the last 30 min of the feed restriction; EE_{act} = energy expenditure for physical activity; DIT = diet-induced thermogenesis.

²P-values from ANOVA analysis.

³P-value from SLICE test.

Table 3. Least squares means and pooled standard error (PSE) of metabolic efficiency measures of dairy cows with low (low-Meff, n = 9) and high (high-Meff, n = 9) metabolic efficiency at wk 5, 13, and 42 postpartum

Item ¹	Wk 5				Wk 13				Wk 42				P-value ²		
	Low-Meff	High-Meff	PSE	P-value ³	Low-Meff	High-Meff	PSE	P-value ³	Low-Meff	High-Meff	PSE	P-value ³	Group	Time	Group × time
Regression data															
Constant a	−516.2	89.5			−446.1	71.8			−459.0	62.4			—	—	—
Coefficient b	0.695	0.06			0.650	0.04			0.622	0.04			—	—	—
Coefficient c	−0.838	0.04			−0.729	0.06			−0.842	0.06			—	—	—
R ² _{adj}	0.95				0.95				0.95				—	—	—
ME _m /mBW, kJ/(kg ^{0.75} d)	743				686				738				—	—	—
k _{it} (from equation [18])	0.83	0.85	0.02	0.66	0.73	0.73	0.03	0.92	0.97	0.83	0.27	0.84	0.31	<0.01	0.56
ME _{IM} /mBW, kJ/(kg ^{0.75} d)	671	350	37	<0.01	774	674	66	0.30	698	552	75	0.18	0.01	<0.01	0.11
ECM _{int} , kg/d	17.2	9.0	1.0	<0.01	18.4	15.8	1.5	0.23	17.2	13.5	1.7	0.15	<0.01	0.01	0.08
ECM _{iss} , kg/d	14.5	23.9	1.9	<0.01	9.3	12.9	1.0	<0.05	5.1	8.6	1.6	0.13	<0.01	<0.01	0.20
ECMe _{int} /mBW, kJ/(kg ^{0.75} d)	466	243	26	<0.01	503	438	43	0.30	434	343	46	0.18	0.01	<0.01	0.07
ECMe _{iss} /mBW, kJ/(kg ^{0.75} d)	397	655	54	<0.01	256	357	28	<0.05	125	217	39	0.11	<0.01	<0.01	0.20
ECMe _{int} /ECMe _{iss}	1.45	0.38	0.19	<0.01	2.32	1.44	0.40	0.13	2.89	2.17	0.56	0.36	<0.01	<0.01	0.91
EEM _{int} /mBW, kJ/(kg ^{0.75} d)	204	107	11	<0.01	271	236	23	0.30	264	209	28	0.18	<0.05	<0.01	0.21
EEM _{iss} /mBW, kJ/(kg ^{0.75} d)	83	121	17	0.12	99	131	14	0.12	15	41	13	0.19	<0.05	<0.01	0.92
(with k _{it} from equation [9])															
EEM _{iss} /mBW, kJ/(kg ^{0.75} d)	78	126	10	<0.01	96	132	10	<0.05	23	40	7	0.08	<0.01	<0.01	0.22
(with k _{it} from equation [18])															
ME _m + EEM _{int} + EEM _{iss}	1,030	971	18	<0.05	1,056	1,053	23	0.92	1,007	978	32	0.53	0.27	<0.01	0.27
EE _m /mBW, kJ/(kg ^{0.75} d)	1,030	971	19	<0.05	1,056	1,053	24	0.93	1,014	988	32	0.56	0.30	<0.01	0.32

¹Coefficient b provides an estimate of the efficiency of ME used for ECM production; coefficient c is an estimate of the efficiency of tissue energy mobilization for ECM production; ME_m = metabolizable energy for maintenance (calculated from −a/b, estimate of ME_m/mBW); mBW = metabolic body weight; MEIM = metabolizable energy intake for milk production; ECMe = milk energy; ECM_{int} = energy-corrected milk yield from feed intake; ECM_{iss} = energy-corrected milk yield from mobilized tissue; ECM_{int} = milk energy from feed intake; ECM_{iss} = milk energy from mobilized tissue; EEM_{int} = energy expenditure of milk production from feed intake; EEM_{int} = energy expenditure of milk production from mobilized tissue; EE = energy expenditure; R²_{adj} = adjusted R² value; k_h = efficiency of tissue utilization for milk synthesis.

²P-values from ANOVA analysis.

³P-value from SLICE test.

Table 4. Least squares means and pooled standard error (PSE) of plasma metabolite concentrations of dairy cows with low (low-Meff, n = 9) and high (high-Meff, n = 9) metabolic efficiency at wk 5, 13, and 42 postpartum

Item	Wk 5				Wk 13				Wk 42				P-value ¹	
	Low-Meff	High-Meff	PSE	P-value ²	Low-Meff	High-Meff	PSE	P-value ²	Low-Meff	High-Meff	PSE	P-value ²	Group	Time
NEFA, ³ μM	514	625	94	0.42	200	171	41	0.61	77	86	10	0.55	0.60	<0.01
Glucose, mM	3.73	3.39	0.17	0.06	3.54	3.23	0.06	<0.05	3.56	3.39	0.13	0.38	<0.05	0.07
Triglyceride, mM	0.14	0.11	0.01	0.19	0.11	0.10	0.01	0.57	0.12	0.12	0.02	0.99	0.32	0.06
BHB, mM	0.52	0.47	0.06	0.55	0.47	0.48	0.04	0.81	0.46	0.53	0.04	0.24	0.82	0.78
														0.37

¹P-values from ANOVA analysis.

²P-value from SLICE test.

³NEFA = nonesterified fatty acids.

mitochondria determines the efficiency of cellular energy production (Salin et al., 2015). There are numerous molecular reasons for the between-animal variation in the P/O ratio and metabolic efficiency, among them the greater uncoupling of the electron transport chain leading to a higher proton leakage across the inner mitochondrial membrane and reactive oxygen species (ROS) production (Salin et al., 2015). Although some of these molecular mechanisms were identified for various monogastric species and beef cattle differing in feed efficiency (Bottje and Carstens, 2009; Cantalapiedra-Hijar et al., 2018), comprehensive studies in dairy cows have not been performed. However, it is tempting to speculate that a lower German breeding value functional herd life of high-Meff cows may be due to higher ROS levels and lower ATP production efficiency.

The higher MEI/mBW of low-Meff cows seem to exist across all stages after calving ($P_{\text{group}} < 0.05$), but was significantly different only at wk 5 pp ($P < 0.01$), whereas ECM was comparable to high-Meff cows during all stages investigated. This result suggests that low-Meff cows, which had higher DMI and thus MEI, use a smaller portion of feed energy for milk production, maintenance, or both. Maintenance energy (ME_m) deduced from regression analysis was not calculated for both groups separately because of the limited number of animals; however, $\text{EE}_{\text{rest}}/\text{mBW}$ as an estimate of maintenance heat production was not different between groups, suggesting that ME_m was also not different among groups. Our finding supports the results of an earlier study in which ME_m values for Holstein Friesian cows, Holstein Friesian crossbreds, and Norwegian dairy cows at various stages of lactation were not different between these breeds and amounted to $686 \pm 16 \text{ kJ}/(\text{kg}^{0.75}\text{d})$ (Dong et al., 2015), which is comparable to the data obtained in the present study. However, the discrepancy between ME_m determined by the regression model (equations [9] and [10]) with the lowest value at wk 13 pp and the experimentally measured EE_{rest} , which was lowest at wk 42 pp, can be explained by the fact that under experimental fasting conditions cows still produce milk and associated heat, whereas the calculation of ME_m presumes that ECM and EB are zero.

It is interesting to note that $\text{EE}_{\text{rest}}/\text{mBW}$ values were greater at wk 5 and 13 than wk 42 pp as these data indicate that fasting heat production and maintenance energy requirement are not constant over the course of lactation. Dong et al. (2015) reported that the energy for maintenance is positively related to ME intake with an increase in ME_m of 62 to 65 $\text{kJ}/(\text{kg}^{0.75}\text{d})$ when ME increases by 1,000 $\text{kJ}/\text{kg}^{0.75}$. Other authors reported that maintenance energy is a function of body protein mass rather than BW or energy intake (Agnew and Yan, 2000). Hence, the intensive fat mobilization dur-

ing the first 5 wk of lactation, which is 4 to 10 times greater than the amount of protein mobilization (Kumaragiri et al., 1998), results in a higher body protein content, which should additionally account for an increase in maintenance energy requirements. Moreover, blood fluxes and O_2 consumption in the portal-drained viscera and liver continuously increased from d 11 to 83 after calving (Reynolds et al., 2003), further indicating increasing energy requirements for maintenance. However, various recent energy feeding systems consider ME_m to depend only on BW and energy for physical activity [e.g., NRC (2001), GfE (2004), NorFor (2011), and INRA (2018)], irrespective of the level of tissue mobilization, DMI, or milk production. Thus, particularly in early- and mid-lactation stages, energy requirements for maintenance of high-producing dairy cows seem to be underestimated and EB overestimated (Dong et al., 2015).

Thermic and CH_4 Energy Losses

The low-Meff cows had greater DIT and heat production (EE/mBW) in early lactation, suggesting that the thermic effect of feed and greater metabolic heat losses account for the smaller portion of MEI available for milk production and thus the lower FCE in low-Meff cows. Comparably, low-FCE beef cattle had greater heat production and 32% higher heat increment of feeding (equals to DIT) than high-FCE cattle (Nkrumah et al., 2006), and low-FCE cows had greater heat production than cows with a higher FCE (Arndt et al. (2015). Therefore, low-FCE animals seem to be more prone to suffer from high ambient temperatures than high-FCE counterparts in times of hot weather periods. In addition, low-Meff cows had higher CH_4 production and CH_4 /mBW than high-Meff cows, again indicating that a smaller portion of ingested feed was available for milk production in these animals. Comparable to our results, Nkrumah et al. (2006) and Arndt et al. (2015) also found less CH_4 energy losses in high-FCE cows and low-RFI cattle, respectively. On the other hand, CH_4 yield as well as rumen acetate, propionate, and butyrate concentrations (data not shown) did not differ between high-Meff and low-Meff cows in the present study, indicating that the ruminal fermentation characteristics were not different between efficiency groups. This conclusion is supported by the study of Potts et al. (2017), who described that the digestive ability of low-RFI and high-RFI cows on a diet containing 30.1% starch per kg of DM is not different between groups and the starch level fed in the present study (24.7%) was only marginally smaller. Our conclusion further agrees with Arndt et al. (2015), stating that the marginally lower digestive efficiency of high-FCE cows in mid and

late lactation is more than compensated for by greater metabolic efficiencies.

Energy Partitioning for Milk Production

Based on equation [9], we calculated the efficiency of ME used for milk production (k_l) and found decreasing values from wk 5 to 42 pp. Our result is line with a the study of Kirkland and Gordon (2001) who reported that early lactating cows partitioned a significantly greater proportion of MEI to milk energy compared with cows in mid or late lactation. Interestingly, high-Meff cows utilized less metabolizable energy intake for milk and milk energy production ($MEIM/mBW$ and $ECMe_{int}/mBW$) from wk 5 to 42 ($P_{group} = 0.01$) with the largest difference between efficiency groups at wk 5 pp ($P < 0.01$). Vice versa, high-Meff cows produced a higher portion of ECM from mobilized tissue (ECM_{tiss} and ECM_{tiss}/mBW) compared with low-Meff cows and this difference was predominantly evident until wk 13 after calving ($P < 0.05$). Thus, high-Meff cows prefer utilizing endogenous rather than dietary energy sources, and their greater metabolic efficiency must be determined by differences in fuel selection. One reason for the preferred utilization of, for example, mobilized fat reserves of high-Meff cows is the energy saving for milk fat synthesis. The energy conversion efficiency of feed-derived acetate or butyrate for the production of 1 mol palmitate by the mammary gland amounts to only 75 to 79% relative to endogenous palmitate utilization and requires 15 to 11 mol ATP, respectively (Bergner and Hoffmann, 1996). However, high-Meff and low-Meff cows did not significantly differ in milk fat and plasma NEFA concentrations, but the latter declined from wk 5 to 42, suggesting that endogenous fat utilization is important for milk fat synthesis for both groups particularly in early lactation. Besides, the oxidation of fat compared with carbohydrate yields approximately 15% more ATP, which is required for de novo syntheses (Salin et al., 2015).

Other energy-saving mechanisms than endogenous fat utilization must cause the difference in tissue utilization between efficiency groups. High-Meff cows tended to have lower plasma glucose concentrations at wk 5 pp than low-Meff cows. Whether high-Meff cows had a lower glycogen reservoir, less de novo glucose synthesis, or used more glucose for energy and milk production can only be speculated. The glycogen reserves primarily stored in muscle and liver become exhausted within the first 40 d of lactation (Schäff et al., 2012) and the mobilization of endogenous glucogenic substrates, primarily lactate, glycerol, and AA, gain importance for gluconeogenesis (Aschenbach et al., 2010). The energy conversion efficiency of glucose converted to 1 mol pal-

mitate is greater than 85% and requires only 7 mol ATP (Bergner and Hoffmann, 1996). In addition, the synthesis of casein from AA and glucose requires 18% less energy than from AA and feed-derived propionate (0.26 vs. 0.34 kJ/kg of casein; Bergner and Hoffmann, 1996). Thus, the superior utilization of endogenous energy reserves, particularly of fat and endogenous glucogenic substrates, for milk production early after calving indicates an energy-sparing mechanism making high-Meff cows metabolically more efficient. The ingestion of less feed but greater use of tissue energy early after calving may be an evolutionary conserved advantage for the calf receiving more energy from the dam but also compromises health, immunity, and EB of the mother, and these compromises are lifted when the calf reduces suckling. Interestingly, dairy cows with a deeper negative EB in early lactation usually ingest less feed and have lower plasma glucose concentrations than cows ingesting more feed but in a less pronounced negative EB (Kuhla et al., 2016). However, it seems surprising that cows in both groups are still in a mild negative EB at wk 42 pp, although their BW increased by 66 to 87 kg relative to wk 13 pp. This apparent discrepancy can be explained by the greater rumen-intestinal fill at wk 42. At this time point, cows had a higher DMI compared with wk 13. Additionally, the loss of BW after the 24-h feed restriction amounted to 69 to 93 kg, which is comparable to the BW increase relative to wk 13. Interestingly, high-Meff cows lost more BW during feed restriction, suggesting that metabolically efficient cows have a higher propensity for tissue mobilization than low-Meff cows. Whether cow groups differ also in their rumen-intestinal volume can only be speculated. However, although the amount of tissue energy for ECM production was greater in high-Meff cows, the efficiency of tissue utilization for milk synthesis (k_{lt}) was not. Constant efficiency of utilization of mobilized tissue ranging between 0.82 and 0.84 was documented earlier by comparing different feeding systems (Agnew et al., 2003).

Similarities and Controversy Between Feed and Metabolic Efficiency

Most definitions of feed efficiency account for the instantaneous use of feed for product generation. As such, dairy cows with low DMI and high ECM yield would account as highly feed efficient (high FCE, low RFI, or low RMEI value), regardless of the source of energy originating either from tissue reserves or feed. In the present study, we identified high-Meff cows possessing a higher FCE and lower RMEI value to produce less methane than low-Meff cows at wk 5 and 13 pp. Therefore, selecting low CH_4 emitting animals in an

attempt to select for greater feed efficiency must be carefully considered as this may result in greater apparent feed and metabolic efficiency with a lower portion of feed energy converted into milk.

CONCLUSIONS

The current study demonstrated that by using MEI, O_2 , and CO_2 , each normalized to mBW, divergent metabolic efficient cows can be distinguished by a cluster approach. At a comparable level of ECM yield, the greater metabolic efficiency of high-Meff cows can be attributed to lower energy losses by heat and methane production, greater utilization of energy from body reserves, and proportionally less utilization of feed energy for milk synthesis as compared with low-Meff cows. Whereas these differences were primarily evident during the early lactation period up to wk 13 pp, they did not persist until wk 42 after calving, indicating that the individual metabolic efficiency trait is not persistent across the whole lactation cycle. Different metabolic efficiency traits could not be assigned to differences in metabolizable energy for maintenance (ME_m) or the efficiency of tissue utilization for milk synthesis (k_{lt}). Future studies should explore whether the degree of metabolic efficiency of dairy cows is determined by the mitochondrial efficiency of ATP production, uncoupling of the electron transport chain, and associated ROS production, and examine if differences in these traits account for the tendency of lower German relative breeding value functional herd life as an indicator of productive lifespan of high-Meff cows.

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