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# Milk somatic cell count and its relationship with feed efficiency, and with GreenFeed-estimated methane emission and energy partitioning variables in Nordic Red cows

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# HIGHLIGHTS

• Higher somatic cell counts (SCC) were associated with reductions in milk yield and efficiency of metabolizable energy use for lactation in Nordic Red cows.

- The reduction in efficiency was primarily due to an increase in heat energy losses.
- Adverse effects on energy utilization efficiency were apparent above 74 000 cells/mL.
- Rising SCC levels were linked to higher methane emission intensity.

#### ARTICLE INFO

*Keywords:* Feed efficiency Heat production Methane emission Somatic cell count

# ABSTRACT

The objective of this study was to evaluate the relationship of somatic cell count (SCC) with production efficiency, energy partitioning, and methane emission in Nordic Red cows. Data were obtained from 10 previously conducted experiments consisting of 3 milk production trials and 7 GreenFeed (GF) studies, with available information on SCC, body weight (BW), milk production and feed intake. The complete data set consisted of a total of 924 cow/period observations from 265 cows. A subset of 150 cow/period observations from three of the GF studies, with available data on digestibility, and heat production were used for analysing energy partitioning variables. All measurements were made on cows fed diets based on grass silage with a range of protein and energy supplements. Production efficiency and energy partitioning variables were evaluated by mixed-model regression in SAS (SAS Institute Inc., Cary, NC). with the natural logarithm of SCC (InSCC) treated as a fixed variable. Experiment (Exp), diet(Exp), and period(Exp) were included as random effects, allowing their effects to be excluded from the fixed-effect estimates. Additionally, segmented regression (PROC NLMIXED in SAS) was applied to determine SCC thresholds affecting feed efficiency variables. An increase in InSCC showed a negative relationship with milk lactose concentration, milk yield, energy corrected milk (ECM), residual ECM, and feed conversion efficiency. Conversely, milk protein concentration increased with rising InSCC. A trend towards increased BW was noted with rising InSCC. Methane energy intensity and heat production increased while the efficiency of metabolizable energy (ME) use for lactation  $(k_l)$  decreased with elevated lnSCC. We identified SCC thresholds of 40 000 cells/mL for heat production (HP) and 74 000 cells/mL for k<sub>b</sub> indicating that HP increases at an earlier stage of infection, while a more advanced infection is required to impair energy utilization efficiency. These findings underscore the importance of early SCC management to sustain production, minimize energy losses, and enhance feed efficiency and overall dairy sustainability.

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#### 1. Introduction

The importance of improved feed efficiency (FE) for the future sustainability of milk production is well recognized by the dairy industry, scientific community, and policy makers around the world, both to maintain profitability and to reduce greenhouse gas production and nutrient losses to the environment (Connor 2015; Løvendahl et al., 2018). As feed cost is the greatest single component of total milk production costs, increase of FE provides a higher income over feed cost for the farmer (Connor 2015). Feed efficiency is mainly influenced by production level and body weight (BW) via the dilution of maintenance (Bauman et al., 1985), such that a greater proportion of metabolizable energy (ME) intake is channelled toward milk production instead of maintenance as production and feed intake increase. This principle becomes evident when examining energy metabolism in controlled settings like respiration chambers, where milk energy output corrected for body energy balance (EB) varies between individual cows fed the same ME intake per metabolic BW (MBW; Agnew et al., 1998). Although the between-cow variation in FE hinges primarily on energy partitioning through ME use above maintenance (Bauman et al., 1985; Agnew et al., 1998), its underpinnings remain inadequately documented. Notably, it is intrinsically associated with animal health factors such as the immune system and inflammatory responses (Zebeli and Ametaj, 2009). The health of a cow can cause changes to DM intake and milk yield (Ballou, 2012), which in turn have a significant impact on FE (Kvidera et al., 2017;Bach et al. 2020).

Mastitis is one of the most common inflammatory diseases within dairy cow production (Gomes and Henriques, 2016). Clinical mastitis (CM) presents distinct symptoms such as udder inflammation and altered milk composition. Subclinical mastitis (SCM) can sometimes present visible signs but not always; nevertheless, it still poses significant negative consequences even in the absence of any noticeable udder abnormalities. An on-going SCM, identified through somatic cell count (SCC), is associated with an increased risk of developing CM (Steeneveld et al., 2008), reduced milk production (Huijps et al., 2008), compromised product quality, and an overall reduction in farm productivity. Although cows with SCC exceeding 200 000 cells/mL are particularly prone to experiencing milk losses (Hagnestam-Nielsen et al., 2009; Potter et al., 2018), very low SCC levels may negatively impact production due to potential udder damage (Franzoi et al., 2020). Indeed, milk losses (2.23 kg/d) associated with low SCC (< 6 250 cells/mL) have been reported (Franzoi et al., 2020) to be even greater than those (1.6 kg/d) linked to high SCC in some cases (Potter et al., 2018), highlighting the complexity of SCC dynamics in dairy cows.

Considering the responses to infection as well as the decreased dilution of maintenance due to a lower milk yield (Bauman et al., 1985), mastitis emerges as a potential factor that may affect the FE of a cow. Arndt et al. (2015) reported a four-fold disparity in SCC values between cows categorized under low and high feed conversion efficiency (FCE) brackets and attributed this to increased energy demands for immune functions linked to SCM. An apparent positive correlation between SCC and residual feed intake (RFI), as illustrated by Xi et al. (2016) accentuates the potential of SCC in elucidating individual cow variability in FE. Moreover, the estimated parallel increase in methane (CH<sub>4</sub>) emission and CH<sub>4</sub> intensity with levels of SCC reported by Potter et al. (2018) highlights the potential environmental consequences of mastitis, bringing forth concerns that extend beyond the scope of milk production. To the best of our knowledge, a recent study by Huhtanen and Bayat (2024) is the first to investigate the relationship between individual cow enteric CH4 emission and SCC, using respiration chambers for CH<sub>4</sub> measurements. However, there is a need to extend this research using more widely adopted CH<sub>4</sub> estimation techniques such as the GreenFeed (C-Lock Inc. Rapid City, SD), to better assess the on-farm environmental costs of mastitis across national herds. Additionally, the relationship between SCC and FE as well as energy partitioning have only been marginally reported. Hence, the objective of the present study

was to test the hypothesis that increased SCC negatively impacts not only milk yield but also FE, energy partitioning, and environmental emissions.

# 2. Materials and methods

#### 2.1. Data collection

Evaluations were performed using data collected from milkproduction trials and studies measuring CH4 and CO2 production with or without O<sub>2</sub> consumption using the GreenFeed (GF) system (C-Lock Inc., Rapid City, SD) in lactating Nordic Red cows. Most of these studies have been published in peer-reviewed scientific journals, and their references are presented in Table 1. For a study to be included, a minimum pre-condition was the availability of data on SCC, production parameters [dry matter intake (DMI), milk production and composition], and BW. The two data sets from 10 studies originally consisted of 265 Nordic Red cows with 936 cow/period observations, along with an additional 36 cow/period observations from 12 Holstein cows. However, to ensure an only Nordic Red data set, these 36 cow/period observations from the 12 Holstein cows were removed. Additionally, 12 outlier observations were detected and excluded based on the Anderson-Darling test (Sauvant et al., 2008), leaving a final analysed data set of 265 Nordic Red cows with 924 cow/period observations. The cow/period observations were considered as the experimental unit.

The production data set was sourced from 2 full lactation studies conducted at Kungsängen (Patel et al., 2017) and Lövsta (Karlsson et al., 2020) research stations of the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden, and 1 unpublished change-over study at the Natural Resources Institute Finland (LUKE) research barn in Jokioinen, Finland. It included 13 diets, 123 cows, and 420 cow/period observations after removing Holstein cows from the study of Karlsson et al. (2020).

The GreenFeed data set was derived from 6 change-over studies (Cabezas-Garcia et al., 2017; Karlsson et al., 2019; Chagas et al., 2021; Fant et al., 2021; Pang et al., 2021; Ramin et al., 2021) and 1 continuous study spanning early- to mid-lactation (1 to 126 DIM; Guinguina et al., 2021a, 2021b) conducted at Röbäcksdalen research station (SLU, Umeå). This data set comprised 25 diets, 142 cows, and 504 cow/period observations after removing statistical outliers. Not all GF studies had data on  $O_2$  consumption for the estimation of HP. Therefore, only three studies (Fant et al., 2021; Guinguina et al., 2021a, 2021b; Pang et al., 2021) including 150 cow/period observations from 45 Nordic Red cows were used to analyse the relationship of SCC with energy partitioning variables.

The experimental periods of all changeover studies (Cabezas-Garcia et al., 2017; Patel et al., 2017; Karlsson et al., 2019, 2020; Chagas et al., 2021; Fant et al., 2021; Pang et al., 2021; Ramin et al., 2021) lasted 21 or 28 days. Data from 1-50 days in milk (DIM) of the continuous GF study (Guinguina et al., 2021a, 2021b) were discarded to have similar range in DIM with the other GF studies and the remaining data were divided into 3 periods of 28 days. For the full lactation studies, the lactation stages were divided into early- (53-120 DIM), mid- (121-210 DIM) and late-(211-305 DIM) to represent periods 1, 2, and 3 within experiment, respectively.

All cows were housed in experimental free stall barns and had free access to water and salt block. The cows from Kungsängen and Lövsta were milked in a robotic AMS system while those from Jokioinen and Umeå were milked in a herring-bone milking parlour twice daily. Individual animal feed intake was recorded daily with Roughage Intake Control feeders (Insentec B.V., Marknesse, The Netherlands) in Umeå and with forage troughs (CRFI, BioControl Norway A/S, Rakkestad, Norway) and concentrate dispensers (FSC400, DeLaval International AB), in Kungsängen and Lövsta. In Jokioinen, daily feed intake was determined as the difference between the feed offered and refusal. For clarification, intake data from cows in the study by Karlsson et al. (2020)

#### Table 1

List of studies, number of diets, individuals, and observations from the data used for the analysis.

Author and year of publication	Location	Type of study	Design	Number of diets	Number of cows		Cow/peri	od observations
					Initial	Used	Initial	Used
Cabezas-Garcia et al., 2017	Röbäcksdalen	GreenFeed	Change-over	4	16	16	63	63
Karlsson et al., 2019	Röbäcksdalen	GreenFeed	Change-over	2	22	22	66	65
Chagas et al., 2021	Röbäcksdalen	GreenFeed	Change-over	4	20	20	80	76
Fant et al., 2021	Röbäcksdalen	GreenFeed <sup>a</sup>	Change-over	4	16	16	58	57
Guinguina et al., 2021a, 2021b	Röbäcksdalen	GreenFeed <sup>a</sup>	Continuous	2	22	22	70	66
Pang et al., 2021	Röbäcksdalen	GreenFeed <sup>a,b</sup>	Change-over	5	30	30	115	113
Ramin et al., 2021	Röbäcksdalen	GreenFeed	Change-over	4	16	16	64	64
Patel et al., 2017	Kungsängen	Production trial	Full lactation	3	71	71	240	240
Karlsson et al., 2020	Lövsta	Production trial	Full lactation	4	36	24	108	72
Unpublished	Jokioinen	Production trial	Change-over	6	28	28	108	108
Total				38	277	265	972	924

<sup>a</sup> GreenFeed studies used in the evaluation of energy partitioning variables.

<sup>b</sup> Only 27 cow/period observations were used from this study in the evaluation of energy partitioning variables.

were the raw data and not corrected values as mentioned therein. The experimental diets were based on grass silage with cereal grains or by-products as energy supplements, and rapeseed meal as protein supplement. The average forage-to-concentrate ratio of the diets ranged from 40:60 to 90:10 on DM basis. Grass silages were produced from primary growth, primary regrowth, and secondary regrowth material, and were wilted before ensiling with application of silage acid-based additive. The concentrates used in each of the studies included cereal grains (barley, wheat, or corn), agro-industrial by-products (molassed or

(kg/d), b<sub>2</sub> = partial regression coefficient of MBW (kg<sup>0.75</sup>), b<sub>3</sub> = partial regression coefficient of  $\Delta BW_p$  (kg/d), b<sub>4</sub> = partial regression coefficient of  $\Delta BW_n$  (kg/d), and  $\epsilon$  = independently and identically distributed random residuals.

Residual ECM yield (RECM) was calculated as the difference between actual ECM (kg/d) and the ECM predicted from a mixed model multiple linear regression, which included DMI, MBW,  $\Delta BW_p$  and  $\Delta BW_n$  as independent variables. Experiment, Diet(Exp), and Period(Exp) were considered as random factors. The regression model was:

$$Predicted \ ECM\left(\frac{kg}{d}\right) = b_0 + b_1 \times DMI + b_2 \times MBW + b_3 \times \ \Delta BW_p + b_4 \times \ \Delta BW_n + \varepsilon$$
(3)

unmolassed sugar beet pulp, citrus pulp, or molasses), protein supplements (soybean meal, canola expeller, or meal), oil supplements, and mineral-vitamin supplements. An additional commercial concentrate was given to cows in the GF studies to lure them to the system. Therefore, the total DMI recorded for cows in the GF studies included concentrate intake from the GF system.

#### 2.2. Calculations

For the complete data set, FE of individual cows was determined through distinct calculations as follows:

Feed conversion efficiency (FCE) : 
$$FCE = ECM \div DMI$$
 (1)

where ECM is energy corrected milk in kg/d and DMI is dry matter intake in kg/d.

Residual feed intake (RFI) was calculated as the difference between actual DMI (kg/d) and predicted DMI. The model used to predict DMI was a mixed model multiple linear regression, with ECM yield, MBW, positive BW change ( $\Delta BW_p$ ), and negative BW change ( $\Delta BW_n$ ) included as independent variables. Adjustments were made for random experiment, period within experiment, and diet within experiment to address potential variations in accuracy of intake records across experiments and differences in growth and lactation for various experimental periods and diets. The regression model was:

Predicted DMI 
$$\left(\frac{kg}{d}\right) = b_0 + b_1 \times ECM + b_2 \times MBW + b_3 \times \Delta BW_p + b_4$$
  
  $\times \Delta BW_n + \varepsilon$  (2)

where  $b_0=$  intercept,  $b_1=$  partial regression coefficient of ECM yield (kg/d),  $b_2=$  partial regression coefficient of MBW,  $b_3=$  partial regression coefficient of  $\Delta BW_p$  (kg/d),  $b_4=$  partial regression coefficient of  $\Delta BW_n$  (kg/d), and  $\epsilon=$  independently and identically distributed random residuals.

For the GF data set, milk energy output (MilkE; MJ/d) was calculated using the ratio, 1 kg of ECM: 3.14 MJ (Sjaunja et al., 1990). Heat production (HP; MJ/d) was calculated from volumes of  $O_2$  consumption (L/d), CO<sub>2</sub> production (L/d), CH<sub>4</sub> production (L/d), and urinary nitrogen excretion (UN, g/d), according to Brouwer (1965).

Energy balance (EB; MJ/d) was calculated for each cow using the following equation:

$$EB = ME \text{ intake} - HP - MilkE$$
(4)

The efficiency of ME use for lactation  $(k_l)$  was calculated according to (AFRC, 1993) as:

$$k_l = (MilkE + a \times EB) \div (ME \ intake - ME_m) \tag{5}$$

where coefficient a = 0.84 if EB < 0 or a = 1/0.95 if EB > 0, and ME<sub>m</sub> is the ME requirement for maintenance (MJ/d) estimated using the following equation from AFRC (1993):

$$ME_m = 0.53 \times (BW \div 1.08)^{0.67} \div (0.35 \times ME \div GE + 0.503)$$
(6)

It should be noted that due to the limited number of observations on  $O_2$  consumption for HP estimates, 150 cow/period observations from three GF studies (Fant et al., 2021; Guinguina et al., 2021a, 2021b; Pang et al., 2021) were used for the analyses of energy partitioning variables.

where  $b_0=\mbox{intercept},\, b_1=\mbox{partial regression coefficient of ECM yield}$ 

# 2.3. Statistical analysis

Statistical analyses were performed with SAS (SAS 9.4 Institute Inc., Cary, NC). Proc MEANS were used for means, min and max values within subsets. The univariate relationship between the natural log-transformed SCC (InSCC) and intake, milk production and composition, FE, as well as energy partitioning variables were analysed by PROC MIXED regression (Littell et al., 2006). The model included InSCC as fixed variable and was defined as follows:

$$Y_{ij} = B_0 + B_1 X_{1ij} + b_0 + b_i X_{1ij} + e_{ij}$$
<sup>(7)</sup>

where  $Y_{ij}$  = the expected value for the dependent variable Y observed at level of <sub>j</sub> of the independent variable X in the study <sub>i</sub>. B<sub>0</sub> = the overall intercept (fixed effect); b<sub>0</sub> = the random effect of study i on the intercept (<sub>i</sub> = 1, ..., 10); b<sub>i</sub> = the random effect of study i on the regression coefficient of Y on X<sub>1</sub> in study i (<sub>i</sub> = 1, ..., 10); B<sub>1</sub> is regression coefficient of Y on X<sub>1</sub> across all studies (fixed effects); X<sub>1ij</sub> = value j of the continuous variable X<sub>1</sub> in study i; and e<sub>ij</sub> = is the residual error. The prediction models included 3 random statements: a random intercept and slope of X<sub>1</sub> with SUBJECT = Exp, a random intercept with SUBJECT = Diet(Exp), a random intercept with SUBJECT = Period(Exp), and a random intercept with SUBJECT = Cow(Exp), using the TYPE = VC (variance components) covariance structure for all random statements. The model incorporated a REPEATED statement since measurements of individual cows were taken repeatedly over time (Period) within experiment [period(Exp)]. A first-order autoregressive covariance structure [AR(1)] was selected based on its Akaike's information criterion being closest to zero, as recommended by Littell et al. (2006). The method = restricted maximum likelihood (REML) statement was used in the PROC MIXED model syntax.

A segmented regression analysis was performed using the non-linear mixed procedure (PROC NLMIXED in SAS) to fit parameter values to the data and to determine the threshold at which SCC levels began impacting feed efficiency variables. The model included lnSCC as fixed variable and experiment as a random effect.

### 3. Results

### 3.1. Descriptive data

Mean diet characteristic and animal variables for both the production and GF studies data set as well as mean energy partitioning variables for the GF data set are shown in Table 2. Both animal and diet parameters showed considerable variation and covered the range in dietary chemical composition relevant to commercial milk production. For both data sets, the range in diet composition and average DMI were similar, whereas average ECM was 1.8 kg/d lower in the GF compared with the production data set. The natural log of SCC for individual cows showed similar coefficient of variation of 0.095 and 0.094 in the production and GF data sets, respectively. The GF data set represented a

Table 2

Description of diet composition, intake, milk production, and energy metabolism variables of cows used in the statistical analysis.

Item	Producti	on studies			GreenFeed studies					
	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max
Diet Composition, g/kg DM										
OM <sup>a</sup>						503	929	10.8	888	950
$CP^{b}$	420	144	14.3	95.1	183	504	156	14.2	130	190
NDF <sup>c</sup>	180	433	25.7	385	490	503	360	33.3	300	471
$\text{DIM}^{d}$	420	155	66.2	53	298	504	126	39.3	52	232
DMI <sup>e</sup> , kg/d	420	21.6	3.40	12.2	31.3	504	21.4	2.70	14.3	27.9
Milk yield, kg/d	420	31.3	6.72	12.9	54.2	504	29.1	5.08	17.1	46.4
ECM <sup>f</sup> yield, kg/d	420	32.7	6.10	14.9	50.1	504	30.9	4.82	18.5	44.8
Milk composition, g/kg										
Fat	420	43.5	5.59	28.3	60.9	504	44.8	5.16	31.1	63.4
Protein	420	34.4	2.93	25.9	43.3	504	35.9	2.98	27.5	43.8
Lactose	420	47.5	1.50	38.8	50.7	504	45.9	1.80	36.2	51.4
SCC <sup>g</sup> , 1000 cells/mL	420	93.5	157	7.2	1663	504	142	265	9	2767
lnSCC <sup>h</sup>	420	10.8	1.03	8.9	14.3	504	11.2	1.05	9.1	14.8
BW <sup>i</sup>	420	625	81.6	443	852	504	611	79.7	433	850
$\Delta BW^{j}$	392	0.22	0.428	-1.18	1.94	389	0.23	0.705	-1.64	2.42
Parity	420	2.0	1.08	1.0	7.0	504	2.2	1.20	1.0	6.0
Digestibility, g/kg DM										
OM <sup>a</sup>						388	724	42.4	612	848
CP <sup>b</sup>						315	649	78.8	310	775
NDF <sup>c</sup>						323	591	70.4	342	780
Energy intake and output, MJ/d										
Gross energy						150	417	64.6	302	598
Digestible energy						150	285	42.4	215	367
Urinary energy						150	24.5	11.7	6.5	51.4
Methane energy						150	24.1	3.74	14.7	36.2
Metabolizable energy						150	235	35.3	144	329
Heat production						150	123	14.8	93	162
Milk energy						150	97.7	15.8	40.2	141
Energy balance						150	9.1	27.6	-64.7	81.9

 $^{a}$  OM = organic matter.

<sup>b</sup> CP = crude protein.

<sup>c</sup> NDF = neutral detergent fibre.

 $^{d}\,$  DIM = days in milk.

<sup>e</sup> DMI = dry matter intake (includes intake of GreenFeed concentrate in GreenFeed studies).

 $^{\rm f}$  ECM = energy corrected milk yield.

 $^{g}$  SCC = somatic cells count.

 $^{\rm h}~lnSCC = natural logarithm of SCC.$ 

<sup>i</sup> BW = body weight.

<sup>j</sup>  $\Delta BW = BW$  change.

wide range of GE intakes and correspondingly large differences in energy outputs from urine,  $CH_4$ , and HP. Methane energy was 24.1 MJ/d on average, with minimum and maximum values of 14.7 and 36.2 MJ/d, respectively. Metabolizable energy intake ranged from 144 to 329 MJ/d and HP from 93 to 162 MJ/d. The range in EB was from -64.7 to 81.9 MJ/d as the data included records of cows with DIM covering 52 to 232 d.

# 3.2. Intake, production, and efficiency

The relationship of lnSCC with intake, milk production, and FE for the complete data set is shown in Table 3. The analysis showed that an increased number of lnSCC had a negative relationship (P < 0.001) with milk lactose concentration, milk yield, ECM, RECM, and FCE. On the other hand, there was a significant increase (P = 0.03) in milk protein concentration with increasing lnSCC. A trend (P = 0.08) towards increased BW and milk fat was noted with rising lnSCC, while no relationships were found between lnSCC and DMI, RFI, or DIM.

# 3.3. Energy partitioning

The relationship between lnSCC and energy partitioning variables from studies conducted in GF is shown in Table 4. Methane energy (CH<sub>4</sub>E) intensity (CH<sub>4</sub>E/MilkE), HP, and HP/GE increased ( $P \le 0.01$ ) with increased lnSCC. Milk energy and the efficiency of ME utilization for milk production ( $k_i$ ) decreased ( $P \le 0.04$ ) with increasing lnSCC.

#### 3.4. Segmented regression

The threshold analysis of the impact of lnSCC on feed efficiency variables is presented in Table 5. Residual ECM remained at 1.1 kg/ d with SCC levels up to 32 000 cells/mL, after which it declined at a steeper rate of  $-0.98 \pm 0.22$ , approximately 1.5 times the slope in the full range of data. For FCE, the corresponding values were 1.37 kg ECM/ kg DMI up to SCC of 33 000 cells/mL above which the slope was  $-0.04 \pm 0.011$ . Heat production (HP) was 122 MJ/d until SCC exceeded 40 000 cells/mL, where the slope increased to  $3.36 \pm 0.978$ , about 1.4-fold compared with the slope in the full range of data. The  $k_l$  remained at 0.65 up to SCC of 74 000 cells/mL before decreasing by  $0.010 \pm 0.005$  per lnSCC. For CH<sub>4</sub>E intensity, the change above a threshold of 38, 000 cells/mL was 15.6 kJ CH<sub>4</sub>E/MJ MilkE.

# 4. Discussion

The results of the present study align with previous research, highlighting the negative relationship between increased SCC and milk yield (Rearte et al., 2022) and milk lactose concentration (Alessio et al., 2021). The findings concerning protein contents also corroborate previous studies (Ahmed et al., 2021). A trend towards increased BW with rising lnSCC could partly be explained by the larger udder size of bigger cows, which can impact milking ease and increase the risk of environmental contamination, leading to bacterial infection (Litwińczuk et al., 2015). In addition, the absolute metabolic demands associated with maintaining a larger body size and producing more milk can contribute to a higher susceptibility to SCM. A unique aspect of the present study is its examination of the relationship between SCC and energy partitioning variables alongside individual cow enteric CH4 emission estimated from the GF system, making it the first to investigate this connection. Although, a recent study by Huhtanen and Bayat (2024) investigated this relationship, their measurements of energy metabolism variables were conducted in respiration chambers. Thus, our study contributes new insights by utilizing the GF technology to explore these dynamics. Higher SCC levels are associated with increased HP and, consequently, reduced energy available for milk production, which could explain the observed decrease in ECM. In general, effectively managing SCC is crucial for improving animal health, feed efficiency, and ensuring the economic and environmental sustainability of dairy herds.

# 4.1. Feed efficiency

Results from the present study suggests that a cow with relatively high SCC (250 000 cells/mL), compared to a cow with a relatively low SCC (50 000 cells/mL) produces 0.05 kg less ECM per kg of DMI (FCE). This loss is outside the range (95 % CI) of values (0.026–0.042) predicted by Potter et al. (2018) for cows at the two levels of SCC. The more pronounced FCE losses in our study could partly be due to sustained DMI even at lower milk production. Olson et al. (2011) also recounted that an incidence of mastitis reduced FE expressed as a ratio of MilkE to gross energy intake in Holstein, Jersey, and reciprocal F<sub>1</sub> crossbred cows.

Only a few studies have addressed the relationship between SCC and RFI in dairy cows, primarily due to a lack of availability of individual feed intake measurements. Xi et al. (2016) found a positive phenotypic correlation between SCC and RFI, suggesting that increased SCC might partly explain variation in the efficiency of feed conversion among cows.

# Table 3

The relationship of InSCC with feed intake, milk production and composition, and feed efficiency in the complete data set (n = 924).

Item	Intercept		Slope			CI		Adj. RMSE		
						Intercept		Slope		
	Estimate	SE	Estimate	SE	P-value	Lower	upper	Lower	upper	
DMI <sup>a</sup> , kg/d	22.4	1.14	-0.07	0.093	0.48	19.8	24.9	-0.25	0.12	1.63
RFI <sup>b</sup> , kg/d	-0.65	1.035	0.06	0.087	0.51	-2.95	1.66	-0.11	0.23	1.50
Milk, kg/d	36.1	1.66	-0.55	0.140	< 0.001	32.4	39.8	-0.83	-0.28	4.13
ECM <sup>c</sup> , kg/d	37.9	1.79	-0.56	0.154	< 0.001	33.9	41.8	-0.86	-0.25	3.11
RECM <sup>d</sup> , kg/d	7.2	1.90	-0.65	0.164	< 0.001	3.0	11.4	-0.97	-0.32	2.79
FCE <sup>e</sup> , kg ECM/kg MI	1.79	0.09	-0.03	0.008	< 0.001	1.57	2.00	-0.04	-0.01	0.15
Milk composition, g/kg										
Fat	41.1	1.90	0.29	0.165	0.08	36.9	45.3	-0.03	0.62	2.99
Protein	33.4	0.90	0.18	0.071	0.01	31.4	35.4	0.04	0.32	1.17
Lactose	51.1	0.62	-0.42	0.046	< 0.001	49.7	52.5	-0.51	-0.33	0.70
BW <sup>f</sup> , kg	598	16.7	2.0	1.13	0.08	560	635	-0.2	4.2	14.4
DIM <sup>g</sup>	134	8.9	0.26	0.418	0.54	115	154	-0.56	1.08	5.35

<sup>a</sup> DMI = dry matter intake.

<sup>b</sup> RFI = residual feed intake.

<sup>c</sup> ECM = energy corrected milk yield.

 $^{d}$  RECM = residual ECM yield.

<sup>e</sup> FCE = feed conversion efficiency.

 $^{f}$  BW = body weight.

<sup>g</sup> DIM = days in milk.

#### Table 4

The relationship of lnSCC with energy partitioning variables from three GreenFeed studies (n = 150).

Item	Intercept		Slope			CI	Adj. RMSE			
					Intercept		Slope			
	Estimate	SE	Estimate	SE	P-value	Lower	Upper	Lower	Upper	
Intake and output, MJ/d										
Gross energy (GE)	466	52.0	-2.9	3.14	0.35	242	690	-9.18	3.30	20.4
Digestible energy (DE)	314	42.3	0.2	2.75	0.96	-224	851	-5.36	5.68	29.2
Methane energy (CH <sub>4</sub> E)	22.6	2.74	0.21	0.237	0.38	10.8	34.4	-0.26	0.68	1.56
Urinary energy (UE)	33	10.2	-0.43	0.404	0.29	-10.7	76.7	-1.24	0.38	3.17
Metabolizable energy (ME)	261	29.5	-1.7	2.14	0.43	134	388	-5.94	2.57	19.8
Heat production (HP)	103	6.9	2.35	0.540	< 0.001	86.0	120	1.26	3.44	4.33
Milk energy (MilkE)	132	13.2	-2.64	1.17	0.03	76	189	-4.99	-0.28	10.1
Energy balance (EB)	20.4	25.2	-0.9	2.15	0.67	-88.1	129	-5.18	3.35	23.5
Energy partitioning, kJ/MJ										
DE/GE	653	31.5	2.2	2.63	0.41	518	789	-3.04	7.40	25.2
CH <sub>4</sub> E/GE	46.3	5.03	0.82	0.420	0.11	34.0	58.6	-0.012	1.64	5.29
CH <sub>4</sub> E/MilkE	118	43.4	11.5	3.82	< 0.01	-68	305	3.9	19.1	42.7
UE/GE	68	17.1	-0.43	0.772	0.58	-5.57	142	-1.97	1.10	7.34
ME/GE	549	31.5	0.82	2.55	0.75	414	685	-4.25	5.90	26.6
HP/GE	217	26.6	7.09	2.29	< 0.01	103	332	2.5	11.7	14.5
$k_l$ a	0.70	0.031	-0.01	0.003	0.03	0.62	0.77	-0.01	-0.0001	0.05

<sup>a</sup>  $k_l$  = Efficiency of ME utilization for milk production [Milk energy at zero energy balance / (ME intake – ME requirement for maintenance)].

# Table 5

Threshold	analysis	of lnSCC	impact o	on feed	efficiency	variables.
	-					

Variable	Intercept		Slope above threshold			Threshold li	Threshold InSCC			Threshold SCC, cells/mL
	Estimate	SE	Estimate	SE	P-value	Estimate	SE	P-value		
RECM <sup>a</sup> , kg/d	1.1	9.44	-0.98	0.22	< 0.01	10.35	0.668	< 0.001	1.75	32 000
FCE <sup>b</sup> , kg ECM/kg DMI	1.37	0.383	-0.04	0.011	< 0.01	10.36	0.514	< 0.001	0.09	33 000
HP <sup>c</sup> , MJ/d	122	31.2	3.36	0.978	0.01	10.40	0.834	< 0.001	8.39	40 000
CH₄E/MilkE <sup>d</sup> , kJ/MJ	279	122.9	15.6	3.80	0.01	10.55	0.484	< 0.001	18.3	38 000
$k_l^{e}$	0.65	0.065	-0.01	0.005	0.04	11.22	0.022	< 0.001	0.01	74 000

<sup>a</sup> RECM = residual energy corrected milk yield.

<sup>b</sup> FCE = feed conversion efficiency expressed as kg ECM per kg dry matter intake (DMI).

<sup>c</sup> HP = heat production.

<sup>d</sup>  $CH_4E/MilkE$  = methane energy intensity expressed as kJ methane per MJ milk energy.

<sup>e</sup> k<sub>l</sub> = Efficiency of metabolizable energy (ME) utilization for milk production [Milk energy at zero energy balance / (ME intake – ME requirement for maintenance)].

Hailemariam et al. (2020) also showed that low-RFI cows had lower SCC compared with high-RFI counterparts. Hou et al. (2012) analysed Bovine SNP genotyping data from cows grouped by high RFI and low-RFI. They (Hou et al., 2012) found that more efficient cows (low RFI) exhibited copy number variations in genes associated with immunity and the inflammation, potentially affecting their ability to elicit a response to an immune challenge. In contrast, Marinho et al. (2021), in their study, found no differences in somatic cell score and mastitis for 400 cows categorized by RFI during early- and mid-lactation. The present study also showed no clear link between InSCC and RFI likely due to similar DMI recorded for all cows irrespective of SCC levels.

We found a negative relationship between InSCC and RECM, suggesting that mastitis may lead to greater economic losses due to cows that yield lower-than-expected ECM. For example, an increase in SCC from 50 000 to 250 000 cells/mL would reduce RECM by 1.0 kg/d. This loss translates to an estimated economic impact of approximately, US \$172 per cow over a 305-day lactation period. These estimates are based on the January 2025 raw milk price in Sweden (Regulation (EU) No 2017/1185 Article 12(a), Annex II.4(a)), and the European Central Bank's average exchange rate for €/US\$ in January 2025. The economic repercussions would even be greater if factors such as the discarding of milk, labour, treatment costs, and culturing tests related to mastitis are considered. Using segmented regression, we identified a threshold SCC level of 32 000 cells/mL, beyond which the negative impacts on RECM became more pronounced. This threshold is lower than the 110 000 cells/mL reported by Huhtanen and Bayat (2024) based on data from two respiration chamber studies with 136 cow/period observations. The

difference in thresholds highlights the complex nature of SCC and its relationship with mastitis, necessitating caution when interpreting results. Whist and Østerås (2007) showed that cows with SCC < 20000cells/mL had the lowest risk of developing CM. However, Franzoi et al. (2020) found that cows with very low SCC (< 6 250 cells/mL) had higher risk of reaching high SCC in a subsequent test day (90 DIM) compared with cows with SCC levels of between 6 250 and 12 500 cells/mL. Additionally, specific mastitic pathogens, such as Streptococcus uberis, Escherichia coli, and coliforms compromise the host immune response, potentially leading to CM even in cases with low SCC (Suriyasathaporn et al., 2000; Peeler et al., 2003; Thompson-Crispi et al., 2014). These findings imply that not all low SCC situations are protective, and the risk varies depending on the specific pathogens involved and the host immune response. Determining an appropriate SCC threshold is therefore, critical to help fine tune the accuracy of RECM loss estimations and assessing the subsequent effect on farm income.

# 4.2. Energy partitioning

In the GF data set of the present study, we found that the relationship between InSCC and HP was strong and positive. At SCC of 250 000 cells/ mL, the predicted HP loss was 3.8 MJ/d higher compared to an SCC of 50 000 cells/mL. This difference is equivalent to 1.2 kg of ECM loss using the average milk energy concentration (3.8 MJ of HP/3.14 MJ/kg of ECM = 1.2 kg/d of ECM; (Sjaunja et al., 1990). The reduction in ECM due to increased HP is equivalent to the mean difference in ECM (1.0 kg/d) between cows in these groups. Hence, the relatively lower ECM production observed in High-SCC than in Low-SCC cows could be attributed to a less efficient metabolic process in converting ME to milk energy. Certainly, we detected a 0.013 difference in  $k_l$  between High-and Low-SCC cows per day. Using the mean DMI of 21.4 kg/d and ME concentration of 11.2 MJ/kg of DM in the GF data set from the above example, the difference of 0.013 in  $k_l$  would give a value of 1.0 kg/d of ECM (also comparable to the observed difference in ECM between Low-and High-SCC cows).

Our findings are in line with Huhtanen and Bayat (2024), who demonstrated a positive relationship between logSCC and HP and negative relationship between logSCC and k<sub>l</sub>. In our study, the SCC threshold for  $k_l$  was 74 000 cells/mL, which is higher than the 40 000 cells/mL threshold for HP. This indicates that energy utilization efficiency may not be immediately affected during the early stages of intramammary infection. However, as infection persists, the cumulative effects of metabolic disruption and heat production losses become more pronounced, reducing energy utilization efficiency. Although our SCC thresholds are lower than those reported by Huhtanen and Bayat (2024), our findings corroborate their conclusion that increased HP due to elevated SCC levels in milk is the main driver of reduced  $k_l$ . The physiological mechanism behind these findings is linked to mastitis-induced inflammation, which triggers a systemic release of inflammatory mediators and negatively impacts the cow's metabolic efficiency (Bach et al. 2020). For instance, pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-1 beta (IL-1 $\beta$ ) can inhibit insulin signalling pathways, impair glucose uptake by peripheral tissues, and promote lipolysis (Egyedy et al., 2022). The immune system responses can lead to an increase in metabolic activity, which require additional energy expenditure, thereby compromising the energy availability for milk production and other physiological processes (Hailemariam et al., 2020). The energy cost of activating the immune system has been reported to be 0.66 g of glucose per kg of metabolic BW per hour in dairy cows (Kvidera et al. 2017). Consequently, cows with increased SCC could expend more energy on immune function than cows with lower SCC, and this could be the likely link between higher SCC and reduced FE observed for Nordic Red cows in the present study. The progressive thresholds for HP and  $k_l$  emphasize the need for early SCC monitoring and intervention to help maintain production, reduce energy losses, and improve overall energy utilization efficiency in lactating Nordic Red cows.

Shuster et al. (1991) measured increased lactose in the urine of cows with mastitis and attributed it to the leakage of lactose out of the alveolus between epithelial cells of the mammary gland. Despite this, they (Shuster et al., 1991) did not find any significant impact of mastitis on urine volume, implying that changes in milk lactose concentration are proportional to changes in lactose excretion in urine. Based on this premise, we would expect an increase in UE/GE in cows with elevated SCC due to their lower milk lactose concentration as evidenced in this retrospective study. However, we detected no relationship between UE/GE and lnSCC, indicating that differences in UE/GE might have no contribution to the variation observed in FE among cows.

#### 4.3. Enteric methane emission

Mitigating CH<sub>4</sub> emissions has long been a priority for the dairy sector. Several strategies have been explored to achieve this goal, with the feeding strategy being the most advanced. Although feeding strategy holds potential for reducing CH<sub>4</sub> emissions of a dairy farm, it may inadvertently result in a trade-off with socio-economic aspects of sustainability (Mostert et al., 2019). This delicate balance between environmental concerns and socio-economic aspects led us to consider the interplay between animal health, particularly mastitis and enteric CH<sub>4</sub> emission, which is a less explored but highly relevant dimension. Earlier studies have started to address the connection between SCC (Gülzari et al., 2018) or CM (Mostert et al., 2019) and greenhouse gas (GHG)

emissions. However, the body of research in this field is still relatively limited. Moreover, literature is limited regarding the relationship between SCC and specific GHG, such as enteric  $CH_4$  emissions at the individual cow level.

In our study, we observed no relationship between lnSCC and both CH<sub>4</sub>E output. This lack of association can be ascribed to the absence of a link between InSCC and GE intake, as well as DE/GE, both of which are important explanatory variables for CH4 production (Ramin and Huhtanen, 2013; Løvendahl et al., 2018; Guinguina et al., 2020). Consequently, the CH4E/GE was not different for cows across various levels of SCC. However, the positive relationship observed between CH4E intensity (kJ/MJ of MilkE) and lnSCC in the present study can be explained by similar CH<sub>4</sub>E output accompanied by lower ECM production in cows with elevated SCC. Potter et al. (2018) estimated an extra 0.34 g of CH<sub>4</sub>/kg milk from a cow with relatively high SCC (250, 000 cells/mL) compared with a cow with relatively low SCC (50, 000 cells/mL). Huhtanen and Bayat (2024) also estimated a similar increase in CH<sub>4</sub> intensity (0.3 g CH<sub>4</sub>/kg ECM) with an increase of SCC from 50 000 to 250 000 cells/mL. Using individual cow CH<sub>4</sub> emission data from the GF system in the present study, we calculated a difference of 18.5 kJ  $CH_4/MJ$  of milk (or 1.07 g of  $CH_4/kg$  of ECM), which is approximately 7.1 % of the annual g CH<sub>4</sub> /kg ECM of a dairy cow in Sweden (Bertilsson, 2016). Using the rate of increase above the threshold of 38 000 cells/mL the increase of CH<sub>4</sub>E intensity was much greater. The estimated difference in CH<sub>4</sub>E intensity between a cow with SCC of 250 000 cells and one at this threshold is 29.3 kJ CH<sub>4</sub>/MJ of milk (or 1.67 g of CH<sub>4</sub>/kg of ECM), which is about 35 % percent more than that estimated from the full range of data. In summary, our study adds to the growing body of literature on the relationship between animal health, particularly SCM, and GHG emissions in dairy farming. These results emphasize the significance of managing diseases like mastitis not only for animal welfare but also for reducing the environmental footprint of dairy production. The implications are clear: healthier cows can potentially translate to lower CH<sub>4</sub> intensity. Further research is warranted to explore strategies for minimizing the impact of mastitis on milk production and CH4 intensity, considering both CM and SCM.

# 5. Conclusions

Nordic Red cows with somatic cell counts (SCC) above 40 000 cells/ mL lose more energy as heat, likely due to increased immune system demands. As SCC exceed 74 000 cells/mL, this inefficiency worsens, reducing the propensity of converting metabolizable energy (ME) into milk even if a cow maintains its feed intake. This reduced efficiency has economic implications as it translates to lower income over feed costs for farmers. Additionally, cows with over 38 000 cells/mL of SCC emit more methane per unit of milk energy output, contributing to the dairy industry's greenhouse gas footprint. Overall, managing SCC and enhancing cow health is not only essential for animal welfare but also pivotal for sustaining dairy farm economics and mitigating methane emission.

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#### CRediT authorship contribution statement

**Abdulai Guinguina:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rebecca Danielsson:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

#### Declaration of competing interest

Authors declare that there is no conflict of interest

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