Estimation of Energy Balance at the Individual and Herd Level Using Blood and Milk Traits in High-Yielding Dairy Cows^{1,2}

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ABSTRACT

This study aimed to estimate individual and herd-level energy balance (EB) using blood and milk traits in 90 multiparous high-yielding Holstein cows, held on a research farm, from wk 1 to 10 postpartum (p.p.) and to investigate the precision of prediction with successively decreased data sets simulating smaller herd sizes and with pooled samples. Dry matter intake, milk yield, and BW were measured daily from parturition through wk 10 p.p. Milk composition was determined 4 times per week, and milk acetone was measured weekly. Blood samples for the determination of metabolites, hormones, electrolytes, and enzyme activities were taken weekly from wk 1 to 10 p.p. between 0730 and 0900. Body condition scores and ultrasonic measurements of backfat thickness and fat depth in the pelvic area were evaluated in wk 1, 4, and 8 p.p. Concentrations of glucose, cholesterol, urea, insulin, insulin-like growth factor-1, triiodothyronine, and thyroxine (T₄) in blood plasma and of lactose and urea in milk were positively correlated with EB, whereas concentrations of nonesterified fatty acids (NEFA), creatinine, albumin, β -hydroxybutyrate, and growth hormone and enzyme activities in blood, and concentrations of fat, protein, fat:lactose ratio, and acetone in milk were negatively correlated with EB. Leptin concentration was not corre-

lated to EB over the first 10 wk p.p. To estimate EB linear mixed-effects, models were developed by backward selection procedures. The most informative traits for estimation of EB were the fat:lactose ratio in milk and NEFA and T₄ concentrations in blood. The precision of estimation of EB in individual cows was low. Using blood in addition to milk traits did not result in higher precision of estimation of herd-level EB, and decreasing sample sizes considerably lowered the precision of EB prediction. Estimation of overall mean herd-level EB over the first 10 wk p.p. using pooled samples was precise even with small sample sizes, but does not consider the level of EB in particular weeks. In conclusion, estimation of herd-level EB at individual weeks using milk traits only has practical implication with herd sizes of ≥ 100 cows if calving is highly seasonal and of ≥ 400 cows if calving is uniformly distributed. Using blood in addition to milk traits does not improve precision of estimation of herd-level EB, regardless of sample size. (**Key words:** energy balance, dairy cow, estimation)

Abbreviation key: AC = acetone, AP = absorbable protein, C30 = cows fed 30% of total DMI as concentrate, C50 = cows fed 50% of total DMI as concentrate, CF = crude fiber, CF-group = concentrate feeding group (C30 or C50), EB = energy balance, ECM = energy corrected milk, GH = growth hormone, GLDH = glutamate dehydrogenase, LDH = lactate dehydrogenase, p.p. = postpartum, T₃ = 3,5,3'-triiodothyronine, T₄ = thyroxine.

INTRODUCTION

High-yielding dairy cows are typically in a state of negative energy balance (EB) postpartum (p.p.) because the amount of energy required for maintenance of body tissue functions and milk production exceeds the amount of energy cows can consume. The degree of negative EB in the early p.p. period and the recovery rate from negative EB are critical for health status and productivity. Insufficient energy supply p.p. may result

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in a higher risk for metabolic disorders, e.g., ketosis (Andersson, 1988), in poor reproductive performance, e.g., a delay in onset of ovarian cycle (Butler and Smith, 1989; Staples et al., 1990; Butler, 2000; Reist et al., 2000), in low conception rates, and in elevated occurrence of inflammatory disease, e.g., endometritis (Dohoo and Martin, 1984; Markusfeld, 1984), and mastitis (Suriyasathaporn et al., 2000). However, estimation of energy status under field conditions is difficult, because energy content of the feed depends highly on environmental factors, such as climate, processing, and storage. Furthermore, estimates of DMI are inaccurate because there is variation with physiological state of the individual cow, ambient temperature, photoperiod, feeding strategy, and forage quality (Allen, 2000; Ingvarsen and Andersen, 2000). In addition, digestibility of feed and of energy-yielding substances and the efficiency of their further use depend on many factors. Therefore, assessments of energy ingested at the animal level might provide more reliable information.

Various metabolic and endocrine blood and milk traits, such as NEFA, ketone bodies, insulin, IGF-1, thyroid hormones (Blum et al., 1985; Kunz et al., 1985; Ronge et al., 1988; Lucy et al., 1992; Gustafsson and Emanuelson, 1996), milk fat, protein, lactose, fat:protein ratio, and fat:lactose ratio (Macleod et al., 1984; Grieve et al., 1986; Steen et al., 1996; Heuer et al., 2000), have been shown to be related to EB. Automated blood and milk analysis and estimation of energy status with multivariate models might therefore have potential use in promoting health and productivity of dairy herds.

Based on such observations, the study aimed to estimate individual and herd-level EB using blood and milk traits in high-yielding dairy cows from wk 1 to 10 p.p. Heuer et al. (2000) reported that herd-mean EB can be adequately estimated with milk test-day information in large dairy herds, but that herd size limits the precision of prediction. In Switzerland, herds are rather small. For this reason it was tested, whether in small herds EB can be better estimated when—in addition to milk test-day information—various metabolic, enzymatic, and endocrine blood traits are incorporated into the models for EB estimation. Furthermore, the precision of prediction at herd-level EB with pooled herd samples was investigated.

MATERIALS AND METHODS

Animals, Husbandry, Feeding Plan, Ration Composition, and Calculation of Energy Balance

The experimental procedures followed the Swiss Law on Animal Protection and were approved by the Com-

mittee for the Permission of Animal Experiments of the Canton of Zug, Zug, Switzerland.

Multiparous dairy cows ($n = 90$; 86 Holstein-Friesian and 4 Red Holstein) of parities 2 to 7 were studied from wk 2 antepartum to wk 20 p.p. at the research farm Chamau of the Swiss Federal Institute of Technology, Hünenberg, Switzerland. The herd-mean 305-d milk production was 9434 ± 1067 (mean $\pm$ SD) kg energy-corrected milk (ECM). The animals were housed in a free-stall barn and were held in free-stall groups of 12 cows. Each free-stall group was equipped with 12 mangers for forage and one automatic feeder for concentrate. Calving seasons were spring (April and May) and fall (November and December). Cows calved in four groups of equal size from fall 1998 to spring 2000.

The feeding plan is summarized in Table 1. During the dry period, cows were fed a roughage mix containing 5 MJ NE_L/kg DM. From wk 4 a.p. until calving, cows received the same roughage mix as p.p. containing 6 MJ NE_L/kg DM and, additionally, concentrate in increasing quantities from 0 to 40% of the calculated amount p.p. After parturition, cows were fed a roughage mix ad libitum (6 MJ NE_L/kg DM), which was freshly provided four times per day. Mangers for roughage were fixed on electronic balances. The concentrate was restricted provided by automatic feeders. Two groups (C30 and C50) were formed based on differences in the total amount of concentrate provided. From wk 1 to 10 p.p., C30 cows ($n = 45$) received concentrate in amounts corresponding to 30% of their individual DMI of the previous week and C50 cows ($n = 45$) in amounts corresponding to 50%, respectively. The two groups were balanced for parity, BCS a.p., BW a.p., calving season, and potential for milk production. The cows had access to roughage and concentrate during 24 h. Individual roughage and concentrate intake of each animal were continuously recorded over 24 h during the whole study period. Minerals and vitamins were fed according to calculated needs (Kessler, 1999), and sodium chloride was provided ad libitum.

The compositions of the rations consumed are shown in Table 2. The roughage mix was composed of grass silage, whole plant corn silage, and grass hay and was kept at approximately 6 MJ NE_L/kg DM. The concentrate contained 8 MJ NE_L/kg DM, and its components were individually mixed according to lactation week and concentrate feeding group (C30 or C50) in order to attain the desired ratio between potentially absorbable protein (AP) and NE_L in the total ration for each individual cow (Table 2). Feed was sampled on a weekly basis, and DM content was determined. Feed was analyzed for CP, crude fiber (CF), AP, and NE_L at the Swiss Federal Research Station for Animal Production, Posieux, Switzerland, on a monthly basis (RAP, 1999).

Table 1. Feeding plan.

Stage of lactation	Roughage	Concentrate		AP ³ /NE _L ratio
		C30 ¹	C50 ²	
Drying off to wk 5 a.p. ⁴	5 MJ NE _L ad libitum	— 0 to 40% of quantity p.p. ⁵ in increasing amounts	— 0 to 40% of quantity p.p. ⁵ in increasing amounts	—
Wk 4 to 1 a.p. ⁴	6 MJ NE _L ad libitum	30% of total DMI	50% of total DMI	— gradually changed from 17 to 15 g/MJ
Wk 1 to 10 p.p. ⁵	6 MJ NE _L ad libitum			

¹C30 = 30% of total DMI as concentrate from wk 1 to 10 p.p.⁵²C50 = 50% of total DMI as concentrate from wk 1 to 10 p.p.⁵³AP = Potentially intestinally absorbable protein.⁴a.p. = Antepartum.⁵p.p. = Postpartum.

The BW was determined twice daily by weighing cows on an electronic balance after milking. The EB was calculated over the first 10 wk p.p. from energy-corrected milk yield (ECM), NE_L intake, and BW using the following equation: $EB = [NE_L\text{-intake} - (kg\ ECM \times 3.14 + 0.293 \times kg\ BW^{0.75})]$, according to Jans and Kessler (1999).

Blood Traits

Blood samples were taken from the jugular vein between 0730 and 0900 using evacuated tubes containing dipotassium-EDTA (1.8 g/L) or no anticoagulant, from wk 1 to 10 p.p. on a weekly basis. Blood samples with the anticoagulant were immediately put on ice, whereas tubes without anticoagulant (for recovery of serum) were left at room temperature until clotting was finished (within 30 min). Tubes were then centrifuged for 20 min at 1500 × g. BHBA, sodium, potassium, chlorine, calcium, phosphorus, magnesium, lactate dehydrogenase (LDH), glutamate dehydrogenase (GLDH), and

aspartate aminotransferase (AST) were determined in serum. Glucose, NEFA, cholesterol, creatinine, albumin, urea, insulin, IGF-1, growth hormone (GH), 3,5,3'-triiodothyronine (T₃), and thyroxine (T₄) were determined in plasma. The samples were stored at -20°C until analyzed at the Division of Animal Nutrition and Physiology, University of Berne, Switzerland, as described by Aeberhard et al. (2001b) and Bruckmaier et al. (1998). For the determination of plasma leptin concentrations in duplicates, the specific double-antibody radioimmunoassay described by Delavaud et al. (2000) was followed, with slight modifications. Instead of anti-ovine Ab 7137 leptin antiserum, Ab 8172 was used in a final dilution of 1:15'000. After addition of ¹²⁵I-ovine leptin, the incubation continued for an additional 44 h instead of 20 h. As second antibody, goat anti-rabbit IgG was used instead of ram anti-rabbit IgG. It was diluted 1:50 in horse serum for standard curves, nonspecific binding tubes, and blanks and 1:50 in incubation buffer for the unknown plasma samples.

Table 2. Least squares means ± SD of compositions of rations consumed from wk 1 to 10 p.p.¹

	Roughage	Concentrate		Total ration	
		C30 ²	C50 ³	C30 ²	C50 ³
CP, g/kg	125.0 ± 16.0	247.0 ± 28.1	217.9 ± 19.3	160.2 ± 12.1	165.6 ± 11.2
CF, ⁴ g/kg	230.7 ± 11.8	83.1 ± 5.4	84.1 ± 5.2	187.9 ± 8.9	166.1 ± 13.0
AP, ⁵ g/kg	77.7 ± 6.2	144.9 ± 14.6	132.5 ± 8.8	97.2 ± 5.3	101.7 ± 5.2
NE _L , MJ/kg	5.9 ± 0.3	7.9 ± 0.1	8.0 ± 0.1	6.5 ± 0.2	6.8 ± 0.2
AP ⁵ /NE _L , g/MJ	13.2 ± 0.5	18.3 ± 2.0	16.7 ± 1.2	15.0 ± 0.6	15.0 ± 0.5

¹p.p. = Postpartum.²C30 = 30% of total DMI as concentrate from wk 1 to 10 p.p.¹³C50 = 50% of total DMI as concentrate from wk 1 to 10 p.p.¹⁴CF = Crude fiber.⁵AP = Potentially intestinally absorbable protein.

Milk Traits

Milk yield was measured twice daily. Time of milking was between 0430 and 0630 and between 1530 and 1730. Milk samples for determination of acetone (AC) were taken once per week (Tuesday morning) from wk 1 to 10 p.p., frozen immediately after milking and stored at -20°C until analyzed. Concentrations of AC were determined by flow injection analysis (Marstorp et al., 1983; Reist et al., 2000) at the Division of Animal Nutrition and Physiology, University of Berne, Switzerland. Milk samples for the determination of milk fat, protein, lactose, and urea were taken four times per week (Monday and Wednesday evening, Tuesday and Thursday morning) and analyzed at the laboratory of the Swiss Brown Cattle Breeders' Federation, Zug, Switzerland. ECM was calculated as $[(0.038 \times g \text{ crude fat} + 0.024 \times g \text{ crude protein} + 0.017 \times g \text{ lactose}) \times \text{kg milk}] \div 3.14$. The 305-d standard lactation milk yields were calculated using the coefficients of the Holstein Association of Switzerland, Posieux, Switzerland.

Statistical Analysis and Model Development

Level of significance was set at $P < 0.05$ throughout the paper. S-PLUS 2000 Professional Release (Math-Soft, 1999) statistical software was used. Mixed-effects models were fitted using the NLME 3.4 library (Pinheiro and Bates, 2000) for S-PLUS.

Incomplete test-day records and test-day records of cows under a medical or preventive treatment, which might have influenced metabolic or endocrine status, were omitted. Thus, 859 test-day records of 90 cows from wk 1 to 10 p.p. were available for calculations.

Descriptive statistical analysis revealed that a considerable number of traits was not normally distributed. Therefore, NEFA, creatinine, BHBA, leptin, insulin, IGF-1, GH, T_4 , LDH, GLDH, AST, milk AC, milk fat, milk protein, fat:protein ratio, and fat:lactose ratio were logarithmically transformed prior to analysis. Correlations between EB and the various blood and milk traits were calculated as Pearson's correlations.

Linear mixed-effects models of the form $y_i = X_i \beta + Z_i b_i + \varepsilon_i$, where y_i is the response vector, β is the vector of fixed effects, b_i is the vector of random effects, X_i and Z_i are the fixed effects and random effects regressor matrices, and ε_i is the within-group error vector, were used to estimate EB considering blood and milk traits from wk 1 to 10 p.p. Models ($n = 6$) were fitted from varying basic models by backward elimination procedures, using a type I-error of $P < 0.05$. In each of these basic models, EB was the response variable and the animal was the repeatedly studied random effect, while the composition of the fixed effects, which are the

physically measurable and recordable data to estimate EB, varied (Table 3). The models were fitted as follows:

- Model 1 was fitted from a basic model containing the fixed effects concentrate feeding group (**CF-group**), parity (dichotomized as 2 and ≥ 3), and week of sampling at the categorical scale, and ECM and the different blood and milk traits at the continuous scale.
- Model 2 was derived from Model 1. Further blood and milk traits were eliminated stepwise from Model 1, regardless of the type I-error, apart from the three most informative ones. Elimination was based on the measure of determination (R^2) of the fixed effects. The rationale for this elimination of traits beyond a type I-error of $P < 0.05$ was to reduce expenditures for laboratory analyses with respect to a potential practical application.
- Model 3 was fitted from a basic model containing the determined blood and milk traits only, i.e., information about CF group, parity, sampling week and ECM was omitted.
- Model 4 was derived from Model 3. Further blood and milk traits were eliminated stepwise from Model 3, apart from the three most informative ones, for the same reasons as in Model 2. Again, elimination was based on the R^2 of the fixed effects.
- Model 5 was fitted from a basic model containing the fixed effects CF group, parity (dichotomized as 2 and ≥ 3) and week of sampling at the categorical scale, and ECM and milk traits at the continuous scale. Blood traits were not included in this model.
- Model 6 was fitted from a basic model containing as fixed effects milk traits only, i.e., information about blood traits, CF group, sampling week, parity, and ECM was omitted.

To avoid collinearity, correlation structures of the fixed effects were examined prior to calculation. If the absolute value of the coefficient of correlation between two variables exceeded $r = 0.5$, the variable correlating stronger to EB was incorporated into the basic model and the other was omitted. The final models were crossvalidated and the residual plots of the final models were used as criterion for the model fit regarding homogenous variance assumption. To estimate precision of estimation of EB at the individual animal level, standard deviations (SD) of observed minus estimated EB (residuals) were calculated for the six final models.

Estimation of Herd-Level Energy Balance

Decreasing sample and herd size was simulated by random selection of cow test-days. In the first simulation, monthly test-days were randomly selected over

Table 3. Composition of the fixed effects of the basic models.

	Milk traits	Blood traits	CF-group ¹	Week p.p. ²	Parity	ECM ³
Model 1	•	•	•	•	•	•
Model 2	•	•	•	•	•	•
Model 3	•	•				
Model 4	•	•				
Model 5	•		•	•	•	•
Model 6	•					

¹CF-group = Concentrate feeding group (C50 = 50% of total DMI as concentrate, C30 = 30% of total DMI as concentrate).

²p.p. = Postpartum.

³ECM = Energy-corrected milk yield.

the first 8 wk of lactation according to the current Swiss milk recording system in the field, resulting in data sets of 180 test-days. In the second simulation, one test-day per cow was randomly selected over the first 10 wk of lactation, resulting in data sets of 90 test-days. In the third simulation, one test-day of 40 randomly identified cows was randomly selected over the first 10 wk p.p., resulting in data sets of 40 test-days. Finally, in the fourth simulation, one test-day of 20 randomly identified cows was randomly selected over the first 10 wk p.p., resulting in data sets of 20 test-days. Each simulation was repeated 200 times, which was considered sufficient for stable SD, of the resulting herd means, according to Heuer et al. (2000). The precision of estimation of weekly EB with reduced data sets was expressed in 1 SD. These weekly SD and the average number of cows in the sample based on the 200 runs were computed for each of the four simulations with each of the six final models.

Finally, precision of estimation of mean overall EB from wk 1 to 10 p.p. was computed as described in the precedent section. Pooling of the samples was simulated in order to minimize laboratory expenditures with respect to a possible practical application. By pooling the individual samples of a herd, cow-specific information, e.g., lactation week and CF-group, got lost. Thus, only models 3, 4, and 6, which do not include the fixed-effects week of sampling, CF-group, and ECM, were considered for this part. Again, simulations were repeated 200 times. The precision of prediction of mean overall EB from wk 1 to 10 p.p. with reduced data sets was expressed in 1 SD of the difference between mean EB estimated with all data sets and mean EB predicted with the reduced data sets.

RESULTS

Correlation of Energy Balance with Blood and Milk Traits

Pearson's correlations of blood and milk traits with EB from wk 1 to 10 p.p. are presented in Table 4.

Apart from leptin, each of the determined metabolic, endocrine, and enzymatic blood traits correlated significantly with EB, as well as the determined milk components. Concentrations of NEFA correlated strongest ($r = -0.685$) with EB, followed by concentrations of glucose ($r = 0.457$), BHBA ($r = -0.451$), and T_4 ($r = 0.418$). In milk, the order of correlation was fat:lactose ratio ($r = -0.589$), followed by fat ($r = -0.565$), fat:protein ratio ($r = -0.496$), and AC ($r = -0.410$).

Model Development and Estimation of Individual and Herd-Level Energy Balance

The six final mixed-effects models selected for estimation of EB are presented in Tables 5 to 10 (Models

Table 4. Pearson's correlations of blood and milk traits with energy balance wk 1 to 10 p.p.¹

Trait	r	P
Blood metabolites		
NEFA	-0.685	<0.001
Glucose	0.456	<0.001
β -hydroxybutyrate	-0.451	<0.001
Cholesterol	0.406	<0.001
Creatinine	-0.415	<0.001
Urea	0.259	<0.001
Albumin	-0.137	<0.001
Blood hormones		
Thyroxine	0.418	<0.001
Insulin-like growth factor-1	0.320	<0.001
3,5,3'-Triiodothyronine	0.270	<0.001
Insulin	0.230	<0.001
Growth hormone	-0.217	<0.001
Leptin	-0.027	0.437
Blood enzymes		
Lactate dehydrogenase	-0.199	<0.001
Aspartate amino transferase	-0.154	<0.001
Glutamate dehydrogenase	-0.114	<0.001
Milk traits		
Fat:lactose ratio	-0.589	<0.001
Milk fat	-0.565	<0.001
Fat:protein ratio	-0.496	<0.001
Milk acetone	-0.410	<0.001
Lactose	0.363	<0.001
Milk protein	-0.185	<0.001
Milk urea	0.103	0.002

¹p.p. = Postpartum.

Table 5. Regression coefficients of Model 1[†] selected for estimation of energy balance [MJ NE_L].

Parameter		Coefficient	95% Confidence interval		P
Constant		83.81	39.59	128.03	<0.001
CF-Group ¹	C50 ²	0			
	C30 ³	-14.84	-19.29	-10.38	<0.001
Week 1		0			
Week 2		4.46	1.55	7.36	0.003
Week 3		15.40	12.03	18.77	<0.001
Week 4		17.25	13.60	20.90	<0.001
Week 5		20.23	16.48	23.99	<0.001
Week 6		20.42	16.68	24.17	<0.001
Week 7		21.71	17.94	25.48	<0.001
Week 8		23.10	19.31	26.89	<0.001
Week 9		24.22	20.48	27.95	<0.001
Week 10		24.81	20.99	28.63	<0.001
ECM, ⁴ kg		-1.98	-2.18	-1.77	<0.001
NEFA, μ mol/L, ln		-1.71	-2.99	-0.42	0.010
Creatinine, μ mol/L, ln		-13.47	-23.08	-3.86	0.007
T ₄ , ⁵ nmol/L, ln		7.36	4.27	10.44	<0.001
Fat:lactose ratio milk, ln		-66.12	-72.57	-59.66	<0.001

[†]Cow is selected as random effect. Sample size (n) = 859 test-day records. R² of fixed effects = 0.711, R² of complete model = 0.900.

¹CF-Group = Concentrate-feeding group.

²C30 = 30% of total DMI as concentrate from wk 1 to 10 p.p.⁶

³C50 = 50% of total DMI as concentrate from wk 1 to 10 p.p.⁶

⁴ECM = Energy-corrected milk yield.

⁵T₄ = Thyroxine.

⁶p.p. = Postpartum.

1 to 6, respectively). The regression coefficients, 95% confidence intervals, *P* values, and R² are shown. Residual plots of the six final models are presented in Figure 1, together with the appropriate SD.

Measure of determination of the fixed effects (R² = 0.711) and precision of estimation of individual EB (1 SD = 12.99 MJ NE_L; Figure 1) were highest in the most complete model (Model 1; Table 5), which included the

Table 6. Regression coefficients of Model 2[†] selected for estimation of energy balance [MJ NE_L].

Parameter		Coefficient	95% Confidence interval		P
Constant		24.95	10.14	39.76	0.001
CF-Group ¹	C50 ²	0			
	C30 ³	-15.49	-20.00	-10.97	<0.001
Week 1		0			
Week 2		4.87	2.04	7.70	<0.001
Week 3		16.61	13.43	19.79	<0.001
Week 4		18.78	15.41	22.14	<0.001
Week 5		21.78	18.32	25.24	<0.001
Week 6		21.91	18.45	25.36	<0.001
Week 7		23.04	19.53	26.55	<0.001
Week 8		24.46	20.94	27.98	<0.001
Week 9		25.52	22.02	29.02	<0.001
Week 10		26.19	22.59	29.79	<0.001
ECM, ⁴ kg		-1.98	-2.18	-1.78	<0.001
NEFA, μ mol/L, ln		-2.04	-3.31	-0.76	0.002
T ₄ , ⁵ nmol/L, ln		7.23	4.13	10.33	<0.001
Fat:lactose ratio milk, ln		-66.98	-73.42	-60.54	<0.001

[†]Cow is selected as random effect. Sample size (n) = 859 test-day records. R² of fixed effects = 0.706, R² of complete model = 0.900.

¹CF-Group = Concentrate-feeding group.

²C30 = 30% of total DMI as concentrate from wk 1 to 10 p.p.⁶

³C50 = 50% of total DMI as concentrate from wk 1 to 10 p.p.⁶

⁴ECM = Energy-corrected milk yield.

⁵T₄ = Thyroxine.

⁶p.p. = Postpartum.

Table 7. Regression coefficients of Model 3[†] selected for estimation of energy balance [MJ NE_L].

Parameter	Coefficient	95% Confidence interval		P
Constant	-2.19	-22.69	18.31	0.835
NEFA, $\mu\text{mol/L}$, ln	-5.26	-6.76	-3.76	<0.001
Cholesterol, mmol/L	3.29	2.44	4.14	<0.001
Albumin, g/L	-0.71	-1.16	-0.26	0.002
T ₄ , ¹ nmol/L, ln	9.51	5.50	13.52	<0.001
Fat:lactose ratio milk, ln	-53.11	-60.58	-45.63	<0.001
Milk urea, mmol/L	-4.07	-5.53	-2.60	<0.001

[†]Cow is selected as random effect. Sample size (n) = 859 test-day records. R² of fixed effects = 0.536, R² of complete model = 0.839.

¹T₄ = Thyroxine.

fixed effects CF-group, sampling week, ECM, and all significant blood and milk traits, namely plasma NEFA, creatinine, T₄, and the fat:lactose ratio in milk. Model 2 was derived from Model 1 by stepwise elimination of blood and milk traits, leaving only the three most informative ones: NEFA and T₄ in plasma and the fat:lactose ratio in milk. In Model 2, R² of the fixed effects accounted for 0.706 (Table 6) and 1 SD of observed minus estimated EB for 13.09 MJ NE_L (Figure 1), respectively. Model 3 included as fixed effects blood and milk traits only, i.e., information about CF-group, sampling week, and ECM was omitted. EB was estimated best by plasma NEFA, cholesterol, albumin, and T₄, the fat:lactose ratio in milk, and milk urea. The R² of the fixed effects was 0.536 (Table 7), and precision of estimation was 16.52 MJ NE_L (Figure 1). Model 4 was derived from Model 3 by stepwise elimination of blood and milk traits, leaving only the three most informative ones. These were the same as in Model 2, namely plasma NEFA and T₄ and the fat:lactose ratio in milk. The R² of the fixed effects accounted for 0.524 (Table 8) and 1 SD of the residuals for 16.70 MJ NE_L (Figure 1), respectively. Model 5 included the fixed effects CF-group, sampling week, ECM, the fat:lactose ratio, and milk AC, but no blood traits. The R² of the fixed effects was 0.695 (Table 9), and precision of estimation was 13.39 MJ NE_L (Figure 1). Model 6 included milk protein, urea, and AC and the fat:lactose ratio as fixed effects. The R² of the fixed effects accounted for 0.330 (Table 10) and 1 SD of the residuals for 19.76 MJ NE_L (Figure 1).

Observed and estimated weekly EB with the six final Models and the complete test-days (n = 859) from wk 1 to 10 are presented in Table 11. For the reduced data sets simulating smaller herd sizes, average number of cows in the samples at the individual weeks are specified. Furthermore, precision of predicted herd-mean EB at decreasing sample (n) and herd size is shown.

Differences between observed and estimated EB with the complete data set (n = 859) were small for Models 1, 2, and 5. For Models 3, 4, and 6 these differences were greater, especially in wk 2 p.p. The SD of predicted herd-level EB with reduced data sets were highest in the first wk p.p., tended to decrease toward wk 10 p.p., and generally increased with declining numbers of observations. This loss in precision of estimation with reduced data sets is illustrated in Figure 2. For each of the six final models, observed weekly EB from wk 1 to 10 p.p. is plotted against the observed EB minus 1 SD of the residual EB obtained with the reduced data sets. To correct for the differences between the six models with respect to the deviance between observed and estimated EB at particular weeks, the absolute value of this deviance (weekly residual mean) was further subtracted.

Mean observed and mean estimated EB for Models 3, 4, and 6 over the first 10 wk p.p. are presented in Table 12. Furthermore, SD of mean EB predicted with pooled samples of reduced data sets are depicted for each of these models. Again, a decrease in sample size

Table 8. Regression coefficients of Model 4[†] selected for estimation of energy balance [MJ NE_L].

Parameter	Coefficient	95% Confidence interval		P
Constant	-29.06	-46.77	-11.34	0.001
NEFA, $\mu\text{mol/L}$, ln	-7.68	-9.13	-6.23	<0.001
T ₄ , ¹ nmol/L, ln	12.14	8.37	15.91	<0.001
Fat:lactose ratio milk, ln	-57.34	-64.13	-50.55	<0.001

[†]Cow is selected as random effect. Sample size (n) = 859 test-day records. R² of fixed effects = 0.524, R² of complete model = 0.821.

¹T₄ = Thyroxine.

Table 9. Regression coefficients of Model 5† selected for estimation of energy balance [MJ NE_L].

Parameter		Coefficient	95% Confidence interval		P
Constant		36.60	28.86	44.34	<0.001
CF-Group ¹	C50 ²	0			
	C30 ³	-15.71	-20.31	-11.11	<0.001
Week 1		0			
Week 2		5.37	2.33	8.42	<0.001
Week 3		17.65	14.21	21.09	<0.001
Week 4		21.14	17.47	24.80	<0.001
Week 5		24.61	20.88	28.35	<0.001
Week 6		25.17	21.52	28.83	<0.001
Week 7		26.79	23.17	30.42	<0.001
Week 8		28.35	24.80	31.89	<0.001
Week 9		29.37	25.90	32.85	<0.001
Week 10		30.76	27.32	34.20	<0.001
ECM, ⁴ kg		-2.04	-2.26,	-1.82	<0.001
Fat:lactose ratio milk, ln		-68.48	-74.95	-62.01	<0.001
Milk acetone, mmol/L, ln		-1.30	-2.23	-0.37	0.007

†Cow is selected as random effect. Sample size (n) = 859 test-day records. R² of fixed effects = 0.695, R² of complete model = 0.897.

¹CF-Group = Concentrate-feeding group.

²C30 = 30% of total DMI as concentrate from wk 1 to 10 p.p.⁵

³C50 = 50% of total DMI as concentrate from wk 1 to 10 p.p.⁵

⁴ECM = Energy-corrected milk yield.

⁵p.p. = Postpartum.

or simulated herd size resulted in a loss in precision of prediction.

DISCUSSION

This study aimed to estimate individual and herd-level EB at individual weeks in early lactation using blood and milk traits in high-yielding dairy cows. We analyzed whether herd-level EB can be accurately estimated even in small herds, when various metabolic, enzymatic, and endocrine blood traits were included in addition to milk components into the models for estimation of energy status. Furthermore, this study investigated whether mean-herd EB in early lactation can be predicted by use of pooled blood and milk samples. For this purpose, 90 Holstein cows kept on highly standardized conditions in a research farm were carefully studied over the first 10 wk p.p. Linear mixed-effects models with the cow selected as the repeatedly studied random effect were used for estimation of EB.

Decreased sample sizes and smaller herd sizes were simulated by random selection of test-days. These simulations were repeated 200 times in order to obtain stable SD for EB prediction with successively decreased data sets.

Correlation of Energy Balance with Blood and Milk Traits

Most coefficients of Pearson's correlations between the various blood and milk traits and EB early p.p. were in the well-known directions and reflected the well-orchestrated endocrine changes and metabolic adaptations in early lactation, i.e., enhanced mobilization of depot fat, skeletal muscle breakdown and favored partitioning of absorbed nutrients to the mammary gland to provide sufficient substrates for milk synthesis (Krebs, 1966; Blum et al., 1983; Kunz et al., 1985; Bauman et al., 1988; Ronge et al., 1988; Chilliard et al., 1998; Chilliard, 1999). The negative Pearson's

Table 10. Regression coefficients of Model 6† selected for estimation of energy balance [MJ NE_L].

Parameter	Coefficient	95% Confidence interval		P
Constant	19.38	3.86,	34.90	0.015
Milk protein, g/L, ln	-28.73	-39.49,	-17.98	<0.001
Milk urea, mmol/L	-5.32	-6.94,	-3.69	<0.001
Fat:lactose ratio milk, ln	-78.51	-86.86,	-70.16	<0.001
Milk acetone, mmol/L, ln	-1.83	-2.99,	-0.66	0.002

†Cow is selected as random effect. Sample size (n) = 859 test-day records. R² of fixed effects = 0.330, R² of complete model = 0.802.

Figure 1. Residual plots of the six final models selected for estimation of energy balance (EB) and SD of the residual EB ($n = 859$).

Table 11. Mean observed and residual energy balance (EB, MJ NE_L) and precision of predicted herd-mean EB at decreasing sample (n) and herd size in individual weeks.

	Week postpartum (p.p.)									
	1	2	3	4	5	6	7	8	9	10
Mean observed EB (n = 859)	-36.2	-37.8	-21.9	-19.1	-13.9	-11.1	-7.3	-4.1	-1.1	1.6
Mean residual EB Model 1 (n = 859)	-0.5	-0.4	0.4	0.4	0.4	-0.1	-0.1	-0.1	0.1	0.0
Mean residual EB Model 2 (n = 859)	-0.2	0.0	0.6	0.6	0.5	0.1	0.1	0.1	0.3	0.1
Mean residual EB Model 3 (n = 859)	3.1	-5.7	0.8	-2.0	-1.3	-1.6	-1.3	0.2	2.1	2.4
Mean residual EB Model 4 (n = 859)	0.9	-6.8	-0.3	-2.6	-0.9	-0.4	0.3	2.1	4.2	4.0
Mean residual EB Model 5 (n = 859)	-0.5	-0.4	0.4	0.5	0.4	-0.2	-0.2	-0.2	0.1	0.0
Mean residual EB Model 6 (n = 859)	2.5	-9.2	-3.2	-3.8	-2.1	-0.8	1.0	3.5	5.7	7.8
SD ¹ of predicted herd mean EB:										
2 samples/cow, 90 cows (n = 180)										
Model 1	3.5	2.7	2.8	2.9	2.7	2.6	2.7	2.6		
Model 2	3.4	2.6	2.7	2.9	2.7	2.6	2.7	2.6		
Model 3	2.4	2.0	2.1	2.1	2.0	2.1	1.8	1.4		
Model 4	2.6	2.1	2.4	2.4	2.2	2.6	2.1	1.6		
Model 5	3.4	2.6	2.7	2.8	2.5	2.6	2.6	2.5		
Model 6	3.0	2.2	2.5	2.5	1.9	2.2	2.0	1.6		
1 sample/cow, 90 cows (n = 90)										
Model 1	6.7	5.2	5.1	6.0	5.5	5.3	5.1	5.4	4.7	4.1
Model 2	6.8	5.0	5.0	6.0	5.6	5.3	5.1	5.4	4.7	4.1
Model 3	4.4	3.7	3.8	3.8	3.6	4.1	3.3	2.7	2.8	2.6
Model 4	5.0	3.8	4.5	4.7	4.3	5.1	3.7	3.1	3.0	2.5
Model 5	6.7	5.1	4.9	5.7	5.3	5.1	4.9	5.4	4.5	3.9
Model 6	5.6	4.5	4.3	4.7	3.7	3.8	3.5	3.0	3.4	3.5
1 sample/cow, 40 cows (n = 40)										
Model 1	10.7	9.8	9.1	8.8	9.5	9.1	8.3	8.2	7.5	7.8
Model 2	10.4	9.4	8.9	8.6	9.4	9.0	8.1	7.9	7.3	7.6
Model 3	7.1	6.9	6.6	5.8	6.7	6.5	5.1	4.7	4.4	4.5
Model 4	7.7	7.7	7.8	6.9	7.7	7.8	6.2	5.4	4.6	4.6
Model 5	10.4	9.3	8.6	8.3	8.7	8.6	8.1	7.4	6.8	7.1
Model 6	9.0	8.4	7.7	6.9	6.5	5.9	5.4	5.1	5.0	5.8
1 sample/cow, 20 cows (n = 20)										
Model 1	17.3	11.9	11.3	11.9	12.3	12.0	10.2	11.5	9.9	10.0
Model 2	16.8	11.4	10.9	11.7	12.0	11.9	9.9	11.1	9.7	9.7
Model 3	11.1	8.5	8.0	8.0	8.7	9.1	6.8	6.1	6.1	5.7
Model 4	11.8	9.3	9.1	9.3	9.8	10.9	7.7	7.5	7.0	6.0
Model 5	16.5	11.4	10.6	11.3	11.2	11.2	9.6	10.7	9.1	9.3
Model 6	13.7	10.3	8.9	9.9	8.7	9.1	7.5	6.7	7.9	7.2

¹Standard deviations of 200 predicted herd means obtained by repeated random sampling from a pool of 859 test-day records (90 cows, 10 weekly tests from wk 1 to 10 postpartum).

correlation of EB to milk protein was probably due to an effect of stage of lactation, i.e., a concomitant increase in EB and decrease in milk protein concentration as the colostrum effect disappeared and, therefore, was not contradictory to the positive regression coefficient of milk protein concentration in the multivariate mixed-effects model estimating EB. The same effect explains the negative Pearson's correlation of plasma albumin to EB, while the regression coefficient of albumin in the multivariate mixed-effects model was positive.

Model Development and Estimation of Individual and Herd-Level EB

Different models (n = 6) for estimation of EB using blood and milk traits were developed. In a first step, a best multivariate model with respect to precision of

estimation of individual EB and R² of the fixed effects was developed (Model 1). Further models with a reduced number of fixed effects and, partly, taking into account milk traits only, were developed with respect to practical applicability. Correlation of the fixed effects was examined prior to calculation in order to avoid collinearity. Whenever the absolute value of the coefficient of correlation between two variables exceeded r = 0.5, the variable correlating stronger with EB was incorporated into the basic model and the other was omitted. As a result, T₄ displaced T₃ and the fat:lactose ratio in milk displaced the fat:protein ratio and milk fat; although using the fat:lactose ratio instead of the fat:protein ratio for estimation of EB is unusual. Nevertheless, Steen et al. (1996) demonstrated in Norwegian dairy herds, that high fat:lactose ratios were closely associated with elevated ketone

Figure 2. Herd means of postpartum energy balance (EB): observed and predicted means (PM) minus 1 SD of the means obtained by 200 repeated random selections of data-sets simulating reduced herd size (Table 11). --●-- = observed mean (n = 859); --□-- = PM¹ minus 1 SD (n = 180); ...▲... = PM¹ minus 1 SD (n = 90); -○- = PM¹ minus 1 SD (n = 40); -◆- = PM¹ minus 1 SD (n = 20).

Table 12. Precision of predicted herd-mean energy balance (EB, MJ NE_L) by pooling of individual samples at decreasing sample (n) and herd size in comparison with observed values.

	Weeks	Model 3	Model 4	Model 6
Mean observed EB (n = 687)	1–8	–19.0	–19.0	–19.0
Mean predicted EB (n = 687)	1–8	–18.1	–18.1	–17.5
Mean observed EB (n = 859)	1–10	–15.2	–15.2	–15.2
Mean predicted EB (n = 859)	1–10	–14.8	–15.2	–15.3
SD ¹ of predicted herd mean EB:				
2 samples/cow, 90 cows (n = 180)	1–8	0.6	0.6	0.6
1 sample/cow, 90 cows (n = 90)	1–10	1.5	1.5	1.4
1 sample/cow, 40 cows (n = 40)	1–10	2.3	2.2	2.3
1 sample/cow, 20 cows (n = 20)	1–10	3.4	3.3	3.5

¹Standard deviations of 200 predicted herd means obtained by repeated random sampling from the pool of 859 test-day records (90 cows, 10 weekly tests from wk 1 to 10 postpartum).

body status and, thus, provided evidence that they are connected with the energy status.

In Model 1, the regression coefficients of the fixed effects reflect metabolic adaptation after parturition. Typically, energy status improved considerably with lactation week (Heuer et al., 2000). The T₄ was positively and NEFA, creatinine, ECM, and fat:lactose ratio were negatively associated with EB, which was more negative in C30 compared with C50 cows. The measure of determination of the complete model was high. The fixed effects explained most of the variability of EB, while the cow as random effect accounted only for a small R². Nevertheless, precision of estimation of individual EB indicated in 1 SD of observed minus estimated EB was rather low. In Model 2, stepwise elimination of blood and milk traits (creatinine) leaving the three most informative ones (NEFA, T₄, and fat:lactose ratio) resulted in the same high R² of the complete model, and only in minor decreases in R² of the fixed effects and in the precision of estimation of individual EB, respectively. For Model 1 as well as for Model 2, EB estimated by use of the complete data set (n = 859) was very close to the observed EB. However, decreased sample and herd sizes considerably decreased precision of prediction of herd-level EB, especially at the onset of metabolic adaptation after parturition in wk 1 p.p. A decrease in variance of EB from wk 1 to 10 p.p. and a more stable metabolic state toward wk 10 p.p. might have resulted in an increased precision of prediction.

Models 3 and 4 aimed to estimate EB by using blood and milk traits as the only fixed effects, i.e., information about CF-group, sampling week, and ECM was omitted. In Model 3, NEFA, albumin, fat:lactose ratio, and milk urea were negatively, and cholesterol and T₄ were positively associated with EB, reflecting metabolic adaptation after parturition. Pearson's correlation between milk urea and EB was positive, reflecting an increase of urea concomitant with DMI. However,

a decrease in energy supply results in decreased ammonia fixation by microbes in the rumen as well as an increased AA catabolism in the liver and, consequently, leads to increased urea synthesis (Oltner and Wiktorsson, 1983), as evidenced by the negative association between EB and milk urea. The remaining three most informative traits in Model 4 were the same as in Model 2. In Models 3 and 4, the R² of the complete models were again relatively high. However, the fixed effects, which are the physically measurable and recordable data to estimate EB, explained considerably less of the variability of EB, while the cow as random effect accounted for a greater part of variance of EB compared with Models 1 and 2. Mainly by elimination of the fixed-effect sampling week, EB could be less precisely estimated and the mixed model attributed some of the weekly variation of EB to the cow, explaining why the cow accounted for a greater part of variance of EB in Models 3 and 4. Hence, precision of estimation of individual EB was considerably lower in Models 3 and 4 than in Models 1 and 2. Mean estimated EB with Models 3 and 4 using the complete data set (n = 859) deviated considerably from mean-observed EB, especially in wk 2 and 4 p.p. and, again, toward wk 10 p.p. Again, these deviations from the observed means, which were stronger for Model 4 than for Model 3, were due to the elimination of the fixed-effect week in Models 3 and 4 compared with Models 1 and 2. In Models 1 and 2, the categorical variable sampling week corrected for the curvilinear trend of EB in the course of lactation, while Models 3 and 4 contained exclusively variables at the continuous scale and, therefore, failed to estimate mean EB at each individual week, even when considering the complete data set (n = 859). Once more, precision of prediction decreased considerably with a decrease in sample and herd size, and the increase in precision of EB from wk 1 to wk 10 p.p. can be explained with a decrease in variance of EB

over this period due to a more stable metabolic state toward wk 10 p.p.

In Models 5 and 6, no blood traits were used to estimate EB. Model 5 comprised CF-group, sampling week, and ECM and was therefore similar to Models 1 and 2, while Model 6 comprised milk components and milk AC and, thus, was similar to Models 3 and 4. The regression coefficients of CF-group and ECM were in the same range as in Models 1 and 2. The fat:lactose ratio and milk AC were negatively associated with EB and reflected physiologic processes, e.g., mobilization of body fat, in early lactation (Steen et al., 1996; Aeberhard et al., 2001a). The fixed-effect milk corrected for the excursive progression of EB. Therefore, EB estimated using the complete data set ($n = 859$) with Model 5 was very close to the observed EB at each of the studied weeks, which is similar in Models 1 and 2. The R^2 of the complete model and of the fixed effects was barely lower than those of the models containing blood traits in addition, and precision of estimation of EB at the individual animal level was in the same range as in Models 1 and 2. The EB at decreasing-herd and sample sizes was also very similar to Models 1 and 2. Therefore, use of blood traits in addition to milk traits does not result in higher precision of prediction of individual and herd-level EB, regardless of sample size. In Model 6, negative associations of milk protein, urea, AC, and fat:lactose ratio with EB reflected the physiological processes in early lactation, as discussed in the previous sections. The R^2 of the complete Model and of the fixed effects were lower than in Models 1 to 5, but the cow accounted for considerably more of the variance than in Models 1 to 5. Precision of estimation of individual EB was lowest compared with the other five models. For the same reasons as in Models 3 and 4, estimated EB using the complete data set ($n = 859$) deviated considerably from mean observed EB at several weeks. Apart from the deviation of estimated from observed EB, especially in wk 2 p.p., precision of prediction of EB at herd level at reduced sample and herd size was as good for Model 6 as for any of the other models.

Prediction of mean overall herd-level EB over the first 10 wk of lactation by pooling samples was precise with each of the applicable models for this purpose (Models 3, 4, and 6), even with reduced data sets and smaller herd size. However, samples were selected randomly from wk 1 to 10 p.p. and were therefore uniformly distributed across this period. Accumulation of calvings either at the beginning or at the end of the sampling period would result in a marked loss in precision of prediction. Therefore, distribution of sampling has to be considered. Furthermore, no information about EB at a particular week can be obtained

when pooling the samples. However, estimation of occurrence and degree of EB nadir might be of particular interest for taking management decisions.

CONCLUSIONS

Estimating EB using blood and milk traits has a great practical potential in monitoring nutritional status p.p. of high-yielding dairy cows on a herd level. Model 5, comprising the fixed-effects concentrate feeding strategy and week of lactation at the categorical scale and ECM, fat:lactose ratio, and milk acetone at the continuous scale, offers a useful tool to precisely estimate the early p.p. herd-level energy status at individual weeks. For this purpose the monthly sampled milk test-day records, as provided by several breeding associations, can be used. However, because precision of estimation decreases tremendously with decreasing sample and herd sizes, estimation of herd-level EB at individual weeks is only suitable in large herds, with herd sizes of ≥ 100 cows if calving is highly seasonal and of ≥ 400 cows if calving is uniformly distributed around the year. Estimation of EB at the individual animal level is not precise enough to allow practical application, neither by use of milk traits alone, nor by use of milk and blood traits. Estimation of overall mean herd-level EB over the first 10 wk of lactation using pooled samples is precise. However, information on herd-level EB in particular weeks is lost when samples are pooled. Remarkably, the use of blood traits in addition to milk traits does not result in higher precision of prediction of individual and herd-level EB, regardless of sample size.

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