Prediction of metabolic status of dairy cows in early lactation with on-farm cow data and machine learning algorithms

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ABSTRACT

Metabolic status of dairy cows in early lactation can be evaluated using the concentrations of plasma β -hydroxybutyrate (BHB), free fatty acids (FFA), glucose, insulin, and insulin-like growth factor 1 (IGF-1). These plasma metabolites and metabolic hormones, however, are difficult to measure on farm. Instead, easily obtained on-farm cow data, such as milk production traits, have the potential to predict metabolic status. Here we aimed (1) to investigate whether metabolic status of individual cows in early lactation could be clustered based on their plasma values and (2) to evaluate machine learning algorithms to predict metabolic status using on-farm cow data. Through lactation wk 1 to 7, plasma metabolites and metabolic hormones of 334 cows were measured weekly and used to cluster each cow into 1 of 3 clusters per week. The cluster with the greatest plasma BHB and FFA and the lowest plasma glucose, insulin, and IGF-1 was defined as poor metabolic status; the cluster with the lowest plasma BHB and FFA and the greatest plasma glucose, insulin, and IGF-1 was defined as good metabolic status; and the intermediate cluster was defined as average metabolic status. Most dairy cows were classified as having average or good metabolic status, and a limited number of cows had poor metabolic status (10–50 cows per lactation week). On-farm cow data, including dry period length, parity, milk production traits, and body weight, were used to predict good or average metabolic status with 8 machine learning algorithms. Random Forest (error rate ranging from 12.4 to 22.6%) and Support Vector Machine (SVM; error rate ranging from 12.4 to 20.9%) were the top 2 best-performing algorithms to predict metabolic status using on-farm cow data. Ran-

dom Forest had a higher sensitivity (range: 67.8–82.9% during wk 1 to 7) and negative predictive value (range: 89.5–93.8%) but lower specificity (range: 76.7–88.5%) and positive predictive value (range: 58.1–78.4%) than SVM. In Random Forest, milk yield, fat yield, protein percentage, and lactose yield had important roles in prediction, but their rank of importance differed across lactation weeks. In conclusion, dairy cows could be clustered for metabolic status based on plasma metabolites and metabolic hormones. Moreover, on-farm cow data can predict cows in good or average metabolic status, with Random Forest and SVM performing best of all algorithms.

Key words: energy metabolism, cattle, cluster analysis, Random Forest

INTRODUCTION

In early lactation, high-yielding dairy cows suffer from body fat mobilization (Bell, 1995; Ingvarlsen and Andersen, 2000) and drastic metabolic changes (Grummer, 1993; Drackley, 1999). Effective and accurate detection of metabolic status of dairy cows could benefit cow health and welfare by application of dietary and management strategies. Plasma metabolites and metabolic hormones have been used to indicate metabolic status of cows (Butler, 2003; Bernabucci et al., 2005; Pezeshki et al., 2007). Poor metabolic status is related not only to increased plasma concentration of BHB and free fatty acids (FFA; also referred to as nonesterified fatty acids) and a decreased concentration of metabolic hormones, such as plasma insulin and IGF-1 (Dann et al., 2005; de Feu et al., 2009), but also to an increased incidence of metabolic disorders such as ketosis and fatty liver (Grummer, 1993; Ingvarlsen et al., 2003; Seifi et al., 2011). Moreover, poor metabolic status in early lactation, indicated by elevated plasma nonesterified fatty acids or elevated BHB concentration, was associated with an increased risk for displaced abomasum,

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culling, and reduced reproductive success (as reviewed by McArt et al., 2013). In addition, incidence of metabolic problems in early lactation has been related to an altered lactation curve with a lower peak milk yield and reduced 305-d milk production (Hostens et al., 2012). Blood sampling, however, is an invasive method that has limited applicability on the farm. A possible alternative is to predict metabolic status with on-farm cow data that are easily obtained—for example, BW, milk production traits, and parity. Milk production traits have been used to predict plasma BHB (Nielsen et al., 2005; van Knegsel et al., 2010) and energy status at herd level of cows (Løvendahl et al., 2010). We hypothesize that on-farm cow data can predict metabolic status of dairy cows in early lactation.

The machine learning method uses statistical techniques to predict cow performance or disease events using large data sets (Shahinfar et al., 2012; Hempstalk et al., 2015). On farm, available cow data usually include a series of correlated variables; for example, fat yield is correlated with milk yield (Wilmink, 1987; Xu et al., 2018). Machine learning algorithms, however, can deal with complicated correlations caused by an ever-increasing number of variables (Dormann et al., 2013). For example, Decision Tree has good predictive performance in correlation-based variable selection (Hall, 1999), and Bayesian inference could mitigate the effect of correlations among a set of variables (Liechty et al., 2004). Machine learning algorithms, such as naïve Bayes, random forest, and artificial neural networks (ANN), have been used to predict milk yield (Gianola et al., 2011), breeding values (Shahinfar et al., 2012), and reproductive performance (Shahinfar et al., 2014; Hempstalk et al., 2015) and to identify mastitis (Cavero et al., 2008; Sun et al., 2010) in dairy cows. Additionally, cluster analysis was used to define metabolic status in dairy cows in early lactation (Tremblay et al., 2018; van Hoeij et al., 2019). To our knowledge, however, research on the application of machine learning algorithms to predict metabolic status of dairy cows in early lactation using on-farm cow data has never been published. In this study, we aimed (1) to define metabolic status of dairy cows through cluster analysis based on their plasma metabolites and metabolic hormones during lactation wk 1 to 7 and (2) to predict metabolic status of those cows using on-farm cow data through machine learning algorithms.

MATERIALS AND METHODS

Data and Animals

Data were collected from 2 earlier studies with protocol number 2010026 (study 1; van Knegsel et al., 2014;

Chen et al., 2016) and 2014125 (study 2; van Hoeij et al., 2017). The experimental protocols were approved by the Institutional Animal Care and Use Committee of Wageningen University and were conducted at Dairy Campus research herd (WUR Livestock Research, Lelystad, the Netherlands). In study 1, 168 Holstein-Friesian cows were assigned randomly to 1 of 3 groups with a 0-, 30-, or 60-d dry period (DP): 56 cows with a 0-d DP (20 cows in parity 2; 36 cows in parity ≥ 3), 56 cows with a 30-d DP (20 cows in parity 2; 36 cows in parity ≥ 3), and 56 cows with a 60-d DP (20 cows in parity 2; 36 cows in parity ≥ 3). After 1 lactation within the experiment, 130 cows were monitored for a second lactation within study 1 (Chen et al., 2016). In study 2, 127 Holstein-Friesian cows were assigned randomly to 1 of 2 groups with a 0- or 30-d DP: 84 cows with a 0-d DP (40 cows in parity 2; 44 cows in parity ≥ 3) and 43 cows with a 30-d DP (19 cows in parity 2; 24 cows in parity ≥ 3). During all lactation stages in both studies 1 and 2, cows were housed in a freestall with slatted floor and cubicles and were milked twice daily. Before calving, dry cows were fed a dry cow diet that consisted of grass silage, corn silage, wheat straw, rapeseed meal, urea, and vitamins and minerals (5.4 MJ of NE_L/kg of DM), whereas lactating cows were fed a lactation diet supporting 25 kg of milk. All cows were fed 1 kg/d of glucogenic or lipogenic concentrate from 10 d before the expected calving date. Postpartum, the experimental concentrate supply was increased in increments of 0.5 kg/d until a level of 8.5 kg/d was reached. Details on diet composition were presented earlier (van Knegsel et al., 2014; Chen et al., 2016; van Hoeij et al., 2017).

Measurements

Body weight and milk yield were recorded daily and averaged per week. Milk fat, protein, and lactose percentage and SCC were analyzed per cow per week (ISO, 2013; Qlip, Zutphen, the Netherlands). Fat- and protein-corrected milk (FPCM) was calculated as (CVB, 2011)

$$\text{FPCM (kg)} = (0.337 + 0.116 \times \text{fat percentage} + 0.06 \times \text{protein percentage}) \times \text{milk yield (kg)}.$$

During wk 1 to 7 postpartum, 10 mL of blood was sampled every Thursday morning from the coccygeal vein into evacuated EDTA tubes (Vacuette, Greiner BioOne, Kremsmunster, Austria). Blood samples were kept on ice before centrifugation for plasma isolation ($3,000 \times g$ for 15 min, 4°C). Plasma samples were stored at -20°C before analysis. Plasma analysis has been previously described (Vicari et al., 2008; Graber

Table 1. Number of dairy cows with complete blood and milk observations in studies 1 and 2 through lactation wk 1 to 7

Study ¹	Lactation week						
	1	2	3	4	5	6	7
Study 1							
First lactation	58	89	92	92	89	89	90
Second lactation	71	104	99	99	91	88	86
Study 2	91	122	120	119	121	122	122
Total	220	315	311	310	301	299	298

¹The experimental design, dry period length, and diet in study 1 first lactation were described by van Knegsel et al. (2014), in study 1 second lactation were described by Chen et al. (2016), and in study 2 were described by van Hoeij et al. (2017).

et al., 2012). In short, the concentration of BHB was measured with kit no. RB1007 (Randox Laboratories, Ibach, Switzerland), FFA with kit no. 994-75409 (Wako Chemicals, Neuss, Germany), glucose with kit no. 61269 (BioMerieux, Marcy l’Etoile, France; Graber et al., 2012), insulin with kit no. PI-12K (EMD Millipore Corp., Billerica, MA), and IGF-1 with kit no. A15729 (Beckman Coulter, Fullerton, CA; Vicari et al., 2008).

Data Transformation

Plasma glucose displayed a normal distribution in all lactation weeks. Raw data of FFA, BHB, insulin, and IGF-1 were first log-transformed to correct for skewness.

Principal Component Analysis and Correlation Analysis

Principal component analysis (PCA) was performed to transform the data of 5 plasma metabolites and hormones into several orthogonal principal components (PC; Borcard et al., 2011) for visualization and interpretation purposes. Data were centered and scaled to unit variance before analysis. The number of PC to retain was determined using the “elbow rule” (Johnson and Wichern, 2002). The bi-plot (by 2 dimensions) was used to investigate the patterns of variation of data and visualize the clustering with respect to different metabolic statuses. The correlation coefficient among 5 plasma metabolites and hormones was determined by Pearson correlation.

Cluster Analysis

In the current study, we assumed that the different metabolic status of dairy cows could be grouped through cluster analysis per lactation week. From studies 1 and 2, a total of 220, 315, 311, 310, 301, 299,

and 298 cows with complete records for both blood and milk were used through lactation wk 1, 2, 3, 4, 5, 6, and 7, respectively (Table 1). To assign dairy cows to metabolic status in each lactation week, *k*-means cluster analysis was applied on weekly plasma BHB, FFA, glucose, insulin, and IGF-1. Briefly, prerequisite cluster number *k* was determined using the “average silhouette” method (Rousseeuw, 1987). Then, a set of *n* samples $X_{(1, 2, \dots, i, i + 1, \dots, n)}$ was grouped into different clusters $C_{j(1, 2, \dots, k)}$; the mean (μ_k) of all samples in each cluster was called the cluster centroid. Based on an iteration (maximum number of 10) that puts 1 sample into a cluster each time, the *k*-means algorithm aims to choose the centroid with a minimized within-cluster sum of squared Euclidean distance among each observation. Average values for plasma BHB, FFA, glucose, insulin, and IGF-1 were calculated per cluster per lactation week and over all 7 lactation weeks.

Definition of Metabolic Clusters

Labeling of clusters for poor, average, or good metabolic status was based on average value within a cluster for concentrations of plasma BHB, FFA, glucose, insulin, and IGF-1 relative to the other clusters within the same lactation week. The cluster with the greatest plasma BHB and FFA and the lowest plasma glucose, insulin, and IGF-1 was defined as poor metabolic status; the cluster with the lowest plasma BHB and FFA and the greatest plasma glucose, insulin, and IGF-1 was defined as good metabolic status; and the intermediate cluster was defined as average metabolic status.

Machine Learning Algorithms

Machine learning algorithms were evaluated to predict cows in poor, average, or good metabolic status. Preliminary analysis indicated a limited number of cows in the poor metabolic status group; therefore, we

built further classifiers to predict cows in either good or average metabolic status. In each lactation week, models were trained by 8 machine learning algorithms using on-farm cow data with 13 features, including BW, DP, parity, milk yield, milk yield standard deviation, fat percentage, protein percentage, lactose percentage, FPCM, SCC, fat yield, protein yield, and lactose yield. Features were presented as an average per week, except milk yield, which was included both as an average per week and as a standard deviation of milk yield per week. In the current study, cow in one lactation was regarded as independent of the same cow in another lactation. Evaluating several algorithms on a specific task is a common practice in machine learning because performance of algorithm may depend on features, sample size, structure, and other characteristics of the data set. In this study, 8 widely used machine learning algorithms were evaluated, including Decision Tree, Naïve Bayes, Bayesian Network, Support Vector Machine, ANN, Bootstrap Aggregation, Random Forest, and K-Nearest Neighbor. All methods were used with default parameters. A brief explanation of each algorithm is presented in the Appendix together with the corresponding parameter settings.

Model Evaluation

In each lactation week, models trained with 8 machine learning algorithms were evaluated through 10-fold cross-validation. Briefly, in 10-fold cross-validation, 10% of the samples in the whole data set were kept as a testing data set and the rest of the samples (90%) were used to train the model. Through lactation wk 1 to 7, in total 29, 65, 64, 63, 56, 51, and 51 cows were in the data set with 2 separate lactations. A cow was only used once per loop of 10-fold cross-validation in order to avoid the same cow being in both the test and train data sets at the same time with a different lactation number. One loop ended until all samples were used exactly once in testing. For each machine learning algorithm, each loop of cross-validation repeated 500 times. Error rate is a priori value to evaluate the predictive performance of machine learning algorithms in each lactation week. To predict cows with 2 metabolic statuses, the error rate is defined as the ratio of false positives and false negatives. To predict cows with 3 metabolic statuses,

$$\begin{aligned} \text{error rate} = \\ 1 - (\text{sum of corrected prediction/sample size}) \\ \times 100\%. \end{aligned} \quad [1]$$

Moreover, the sensitivity, specificity, positive predictive value (**PPV**), and negative predictive value (**NPV**) of machine learning algorithms in each lactation week were also presented (Wong and Lim, 2011). Based on preliminary analysis, Random Forest performed well not only in model accuracy but also in sensitivity and NPV. The importance of each feature in Random Forest was investigated by mean decreased Gini (Louppe et al., 2013). Briefly, the importance of feature X_m to predict Y is evaluated by adding up weighted impurity decreases $p(t) \Delta i(s_t, t)$ for all nodes t where X_m is used, averaged over all N_T trees in Random Forest:

$$\text{Imp}(X_m) = \frac{1}{N_T} \sum_T \sum_{t \in T: v(s_t)=X_m} p(t) \Delta i(s_t, t), \quad [2]$$

where X_m is the features used in Random Forest, $p(t)$ is the proportion N_t/N of samples reaching t , and $v(s_t)$ is the variable used in split s_t (Breiman, 2001). A lower Gini (i.e., higher decreased Gini) means that a feature plays a relatively more important role than others to discriminate samples into defined classes.

Data Processing and Software

Multiple comparison analysis was applied with Tukey's honest significant difference test. Data pre-processing and machine learning algorithms were programmed in the R environment (version 3.4.3; <https://www.r-project.org/>), with function "kmeans()" for cluster analysis, function "prcomp()" for PCA, function "cor.test()" for Pearson analysis, package "rpart" for Decision Tree, "neuralnet" for Bayesian Network, "e1071" for Naïve Bayes and Support Vector Machine, "adabag" for Bootstrap Aggregation, and "randomforest" for Random Forest. Figures were plotted in Python (version 3.6; <https://www.python.org/>) using modules "pandas," "matplotlib," and "Seaborn."

RESULTS

PCA and Correlation Analysis

All plasma metabolites and metabolic hormones were correlated (Pearson) with a correlation coefficient (absolute value) <0.61 ($P < 0.05$) or insignificant ($P > 0.05$; Table 2). Figure 1 shows the bi-plot (limited to the first 2 components for the sake of simplicity) from the PCA and presents the explained variation in first PC (range: 47.5–62.6% during wk 1 to 7) and second PC (range: 13.5–18.2%). Inspection of the PCA loadings shows the relative contribution of 5 plasma

Table 2. Pearson coefficients ($P < 0.05$) among the concentrations of plasma free fatty acids (FFA), BHB, glucose, insulin, and IGF-1 of dairy cows through lactation wk 1 to 7

Item	Wk 1				Wk 2				Wk 3			
	FFA	BHB	Glucose	Insulin	FFA	BHB	Glucose	Insulin	FFA	BHB	Glucose	Insulin
BHB	0.52				0.52				0.41			
Glucose	-0.18	-0.36			-0.49	-0.53			-0.48	-0.57		
Insulin	-0.47	-0.20	0.32		-0.38	-0.24	0.48		-0.44	-0.20	0.45	
IGF-1	-0.55	-0.29	0.34	0.58	-0.51	-0.31	0.60	0.50	-0.50	-0.30	0.61	0.45
Item	Wk 4				Wk 5				Wk 6			
	FFA	BHB	Glucose	Insulin	FFA	BHB	Glucose	Insulin	FFA	BHB	Glucose	Insulin
BHB	0.39				0.43				0.39			
Glucose	-0.45	-0.53			-0.43	-0.52			-0.37	-0.48		
Insulin	-0.38	-0.24	0.40		-0.33	-0.17	0.40		-0.20	NS	0.32	
IGF-1	-0.52	-0.16	0.57	0.32	-0.49	-0.25	0.57	0.37	-0.42	-0.22	0.53	0.25
Item	Wk 7											
	FFA	BHB	Glucose	Insulin								
BHB	0.32											
Glucose	-0.38	-0.43										
Insulin	-0.29	-0.17	0.34									
IGF-1	-0.46	-0.18	0.57	0.31								

metabolites and hormones to cluster metabolic status of dairy cows (bi-plot of PCA; Figure 1). Raw data of loading are presented in Supplemental Table S1 (<https://doi.org/10.3168/jds.2018-15791>). The loading pattern of plasma metabolites and hormones is the same across lactation wk 1 to 7.

Definition of Clusters for Metabolic Status

The optimal number of clusters suggested by Average Silhouette was 3 through lactation wk 1 to 7. In each lactation week, the silhouette widths per k numbers in Cluster Analysis are presented in Supplemental Figure

S1 (<https://doi.org/10.3168/jds.2018-15791>). Based on the concentration of plasma metabolites and metabolic hormones, cows in the first, second, and third clusters were defined as having good, average, and poor metabolic status, respectively. Cluster 3, defined as poor metabolic status, had the greatest plasma BHB and FFA but the lowest glucose, insulin, and IGF-1 ($P < 0.05$; Table 3). Conversely, cluster 1, defined as good metabolic status, had the lowest plasma BHB and FFA but the greatest plasma glucose, insulin, and IGF-1 ($P < 0.05$). For all lactation weeks, cluster 2 had a lower plasma BHB and lower plasma FFA than cluster 3 ($P < 0.05$) but also lower plasma insulin, IGF-1, and glucose

Figure 1. Dairy cows clustered for 3 metabolic clusters through lactation wk 1 to 7 through plasma metabolites and metabolic hormones. Metabolic clusters are defined as good (○), average (▲), or poor (×) metabolic status. Percentage in brackets represents error rate to predict cows in good or average metabolic status using on-farm cow data. Error rate of each cow is represented with dot size (small = low error rate; big = high error rate). Cows were plotted as 1 label in a 2-dimensional plot in principal components (PC) analysis; 1st PC and 2nd PC stand for the explained variation by first and second PC in PC analysis, respectively. The arrow line represents the loading of plasma BHB, free fatty acids (FFA), glucose, insulin, and IGF-1 in the first PC in PC analysis through lactation wk 1 to 7.

Table 3. Plasma metabolite and metabolic hormone concentrations for dairy cows in 3 metabolic clusters in lactation wk 1 to 7 postpartum¹

Week	Cluster	No. of cows	BHB (mmol/L)	FFA (mmol/L)	Glucose (mmol/L)	Insulin (μU/mL)	IGF-1 (ng/mL)	MS
1	1	35	0.60 (0.17) ^a	0.12 (0.10) ^a	4.63 (1.27) ^a	31.81 (17.88) ^a	162.9 (55.0) ^a	Good
	2	135	0.58 (0.13) ^a	0.38 (0.22) ^b	3.75 (0.67) ^b	10.34 (4.81) ^b	71.7 (36.6) ^b	Average
	3	50	1.10 (0.36) ^b	0.87 (0.32) ^c	3.25 (0.59) ^c	6.37 (3.44) ^c	33.6 (20.9) ^c	Poor
	Average		0.87 (0.31)	1.10 (0.35)	3.25 (0.58)	12.87 (11.72)	77.6 (11.7)	
2	1	80	0.56 (0.13) ^a	0.14 (0.11) ^a	3.90 (0.29) ^a	18.35 (7.90) ^a	154.3 (69.1) ^a	Good
	2	199	0.75 (0.26) ^b	0.43 (0.21) ^b	3.22 (0.36) ^b	7.96 (3.75) ^b	57.1 (28.9) ^b	Average
	3	36	2.55 (1.25) ^c	0.97 (0.43) ^c	2.55 (0.43) ^c	5.55 (2.52) ^c	32.2 (16.4) ^c	Poor
	Average		0.91 (0.76)	0.42 (0.32)	3.32 (0.53)	10.32 (6.92)	78.9 (61.3)	
3	1	95	0.60 (0.14) ^a	0.13 (0.09) ^a	3.86 (0.29) ^a	18.72 (9.19) ^a	138.9 (49.5) ^a	Good
	2	195	0.80 (0.32) ^b	0.40 (0.19) ^b	3.24 (0.36) ^b	8.86 (4.28) ^b	69.7 (26.6) ^b	Average
	3	21	2.95 (0.91) ^c	0.64 (0.24) ^c	2.48 (0.44) ^c	7.07 (3.39) ^b	47.6 (19.5) ^c	Poor
	Average		0.89 (0.66)	0.33 (0.22)	3.38 (0.51)	11.75 (7.71)	89.4 (48.2)	
4	1	129	0.64 (0.19) ^a	0.12 (0.08) ^a	3.88 (0.3) ^a	19.2 (10.9) ^a	131.9 (41.9) ^a	Good
	2	168	0.79 (0.37) ^b	0.39 (0.20) ^b	3.33 (0.36) ^b	9.70 (4.06) ^b	73.5 (25.8) ^b	Average
	3	13	3.79 (1.25) ^c	0.67 (0.24) ^c	2.47 (0.55) ^c	8.01 (6.58) ^b	52.3 (29.2) ^b	Poor
	Average		0.85 (0.73)	0.28 (0.22)	3.52 (0.49)	13.58 (9.08)	96.9 (44.9)	
5	1	110	0.63 (0.22) ^a	0.11 (0.08) ^a	3.99 (0.32) ^a	22.03 (12.63) ^a	140.4 (41.5) ^a	Good
	2	169	0.71 (0.30) ^a	0.30 (0.16) ^b	3.48 (0.34) ^b	10.37 (4.23) ^b	79.5 (26.4) ^b	Average
	3	22	2.97 (1.54) ^b	0.63 (0.33) ^c	2.72 (0.35) ^c	8.11 (3.83) ^c	53.1 (16.4) ^b	Poor
	Average		0.85 (0.77)	0.26 (0.21)	3.61 (0.48)	14.47 (10.12)	99.8 (45.1)	
6	1	117	0.60 (0.19) ^a	0.11 (0.08) ^a	4.03 (0.28) ^a	20.69 (14.01) ^a	143.4 (40.1) ^a	Good
	2	163	0.67 (0.25) ^a	0.27 (0.16) ^b	3.55 (0.31) ^b	11.64 (5.78) ^b	76.6 (23.9) ^b	Average
	3	19	2.76 (1.27) ^b	0.51 (0.37) ^c	2.97 (0.31) ^c	8.96 (2.44) ^b	62.0 (22.5) ^b	Poor
	Average		0.78 (0.64)	0.22 (0.19)	3.70 (0.42)	15.01 (10.78)	101.8 (45.7)	
7	1	144	0.61 (0.20) ^a	0.09 (0.06) ^a	3.99 (0.28) ^a	20.2 (12.92) ^a	132.8 (37.0) ^a	Good
	2	144	0.69 (0.29) ^a	0.25 (0.14) ^b	3.55 (0.31) ^b	12.02 (5.97) ^b	74.3 (23.6) ^b	Average
	3	10	3.08 (1.25) ^b	0.37 (0.11) ^c	2.97 (0.58) ^c	8.30 (2.97) ^b	68.7 (15.5) ^b	Poor
	Average		0.73 (0.55)	0.18 (0.14)	3.74 (0.4)	15.85 (10.77)	102.4 (42.5)	
Average	1		0.61 (0.18) ^a	0.11 (0.08) ^a	3.98 (0.43) ^a	20.55 (12.38) ^a	140.3 (46.9) ^a	Good
	2		0.72 (0.29) ^b	0.35 (0.20) ^b	3.42 (0.43) ^b	9.99 (4.88) ^b	71.3 (28.3) ^b	Average
	3		2.38 (1.27) ^c	0.75 (0.37) ^c	2.83 (0.57) ^c	7.03 (3.66) ^c	44.2 (23.2) ^c	Poor

^{a-c}Values within a lactation week of dairy cows in different metabolic status with different superscripts differ ($P < 0.05$).

¹Cluster analysis of metabolic status (MS) was based on the concentration of plasma free fatty acids (FFA), BHB, glucose, insulin, and IGF-1. Values represent means (SD in parentheses).

than cluster 1 ($P < 0.05$) and was therefore defined as the cluster of cows with an average metabolic status.

Predictive Performance of Machine Learning Algorithms

To predict cows in either good or average metabolic status, SVM had the lowest error rate in lactation wk 3 to 7 of all algorithms. Random Forest had the lowest error rate in lactation wk 1 and 2 and a slightly higher error rate through lactation wk 3 to 7 (Table 3). The sensitivity and NPV of all algorithms were lower in lactation wk 1 than in other weeks. Random Forest had higher sensitivity (range: 67.8–82.9% during wk 1 to 7) and NPV (range: 89.5–93.8%), and SVM had higher specificity (range: 80.9–93.7%) and PPV (range: 78.8–86.1%; Figure 2). After 500 repetitions in Random Forest, error rate to predict cows in good metabolic status was higher than error rate to predict cows in average metabolic status (Figure 1). In 2-dimensional PCA plots, major incorrectly predicted dots (big dots)

are mainly presented in the border area between good and average metabolic status groups (Figure 1).

Machine learning algorithms to predict cows in poor, average, or good metabolic status were evaluated (Supplemental Table S2, <https://doi.org/10.3168/jds.2018-15791>). The general performance of 8 algorithms decreased when 3 metabolic statuses were included in the prediction compared with 2 metabolic statuses; error rate ranged from 21.36 to 48.7%. Random Forest and SVM were still the best-performing algorithms through all lactation weeks.

Feature Contribution

Random Forest had a lower error rate but also had the highest sensitivity and NPV to predict dairy cows with good metabolic status (Table 4; Figure 2). The contribution of features to predict metabolic status in Random Forest is shown in Figure 3. Through lactation wk 1 to 3, milk yield, fat yield, and protein percentage were more important than other features; in wk 4 and

Figure 2. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of machine learning algorithms to predict cows in good or average metabolic status using on-farm cow data through lactation wk 1 to 7. DT = Decision Tree; NB = Naive Bayes; BN = Bayesian Network; SVM = Support Vector Machine; ANN = Artificial Neural Networks; BA = Bootstrap Aggregation; RF = Random Forest; KNN = K-Nearest Neighbor. The sensitivity and PPV of ANN are not presented in wk 1 to 6 due to averages lower than 0.40 in all lactation weeks, and the specificity and NPV of ANN are not presented due to averages lower than 0.65 in all lactation weeks. Values represent means, and error bars represent SD of 500 repetitions.

Table 4. Error rate (%; SD in parentheses) of machine learning algorithms to predict 2 metabolic statuses (good and average) of dairy cows through lactation wk 1 to 7

Machine learning algorithm	Lactation week							Average
	1	2	3	4	5	6	7	
Decision Tree	20.0 (2.6)	14.6 (1.7)	21.3 (2.2)	21.7 (2.1)	22.0 (2.2)	22.5 (2.1)	23.1 (2.4)	20.7 (3.5)
Naive Bayes	16.6 (1.0)	14.2 (1.0)	15.1 (0.9)	17.0 (1.4)	18.4 (1.2)	20.6 (1.0)	23.6 (1.2)	17.9 (3.2)
Bayesian Network	17.4 (1.5)	13.9 (1.6)	16.8 (1.3)	17.5 (1.6)	20.3 (1.9)	21.5 (1.3)	21.7 (1.5)	19.2 (2.5)
Support Vector Machine	16.4 (1.2)	12.4 (1.3)	15.4 (1.3)	14.1 (1.4)	17.3 (1.2)	19.0 (1.3)	21.1 (1.3)	16.5 (3.0)
Artificial Neural Networks	24.7 (2.2)	36.8 (2.7)	43.7 (3.0)	49.8 (5.0)	44.8 (3.8)	47.1 (4.1)	47.8 (6.3)	43.3 (7.7)
Bootstrap Aggregation	16.7 (1.3)	13.0 (1.2)	17.8 (1.3)	18.4 (1.4)	18.5 (1.3)	20.2 (1.6)	21.6 (1.8)	18.0 (2.9)
Random Forest	15.9 (1.3)	12.4 (1.3)	16.4 (1.3)	16.9 (1.4)	18.4 (1.4)	19.6 (1.4)	21.6 (1.5)	17.6 (3.2)
K-Nearest Neighbor	22.3 (1.7)	19.8 (1.6)	23.4 (1.7)	22.1 (1.6)	27.9 (1.9)	26.4 (1.6)	32.3 (2.0)	24.9 (4.3)
Average	18.2 (3.0)	15.1 (4.8)	18.8 (5.0)	18.5 (4.3)	23.5 (8.9)	24.6 (9.0)	24.2 (5.3)	

5, protein percentage and lactose yield were the most important features to predict metabolic status; and in wk 6 and 7, milk yield, protein percentage, and lactose yield were more important than other features, whereas fat-related features were not as important as in lactation wk 1 to 3.

DISCUSSION

Metabolic Clusters

In the current study, we first aimed to define metabolic status of dairy cows through cluster analysis based on their plasma metabolites and metabolic hormones through lactation wk 1 to 7. It was hypothesized that altered plasma metabolites and metabolic hormones between different clusters could indicate the difference in metabolic status. Moreover, the correlation among these plasma variables was in line with previous studies; for example, greater plasma glucose concentration was negatively correlated with plasma BHB and FFA concentration (Francisco et al., 2002; van Knegsel et al., 2007). In the current study, however, the majority of the correlations between plasma metabolites and metabolic hormones had a trend to get weak from lactation wk 1 to 7. For example, the correlations between plasma FFA and insulin and between plasma IGF-1 and insulin got smaller from wk 1 until 7. Cluster analysis can be affected by variables with high correlation (Hair et al., 2006), which is usually defined as a correlation of above 0.8 or 0.9 (Franke, 2010). The maximum correlation among variables in the current study was 0.61. An alternative could be to perform cluster analysis on selected PC, which are orthogonal and thus uncorrelated (Borcard et al., 2011; Tremblay et al., 2018). The sum explained variation by first and second PC accounted for 65.7 to 76.1% over lactation weeks in the current study (Figure 1). Therefore, when using selected PC (e.g., 2 PC) from PCA in cluster analysis, 2 PC could

account for only 65.7 to 76.1% of explained variation of 5 plasma metabolites and metabolic hormones.

In the current study, metabolic status was a composite trait of plasma BHB, FFA, glucose, insulin, and IGF-1. Cows with poor metabolic status had, on average, a greater BHB concentration than the earlier suggested threshold for subclinical ketosis (>1.2 mmol/L; Duffield and LeBlanc, 2009), or a greater plasma FFA than the suggested threshold for subclinical ketosis (>0.6 mmol/L; Seifi et al., 2011), except in lactation wk 7. Recently, Tremblay et al. (2018) identified plasma values for poor metabolic adaptation syndrome (PMAS) in dairy cows in early lactation. Results of this study are in line with the current study for cows with good metabolic status but not completely for cows with poor metabolic status. In the current study, cows with good metabolic status had, on average, a lower plasma FFA concentration over lactation weeks than the earlier suggested threshold for low PMAS (<0.39 mmol/L; Tremblay et al., 2018). However, cows with poor metabolic status had, on average, a greater plasma FFA concentration in wk 1 and 2 than the earlier suggested threshold for high PMAS (≥ 0.7 mmol/L; Tremblay et al., 2018). The difference between these 2 studies might be related to the fact that PMAS clusters were based on plasma FFA, BHB, and milk fat: protein ratio (Tremblay et al., 2018), whereas in the current study metabolic status was a composite trait based on plasma FFA, BHB, glucose, insulin, and IGF-1. For all lactation weeks, cows with good metabolic status had plasma IGF-1 concentration >100 ng/mL (Lomander et al., 2012) and greater plasma glucose and insulin concentration compared with the other 2 clusters. Moreover, in our study, cluster analysis was done per lactation week, which avoids a possible confounding effect of lactation stage with metabolic status of dairy cows. The implication of this approach is that metabolic health classifications are relative to the other 2 clusters within a lactation week. This could imply

Figure 3. Importance of all features in Random Forest classification through lactation wk 1 to 7. Contribution of features was evaluated by Mean Decreased Gini (Equation 2). DPL = dry period length; FPCM = fat- and protein-corrected milk production; Milk SD = standard deviation of daily milk yield. X-axis represents mean decreased Gini of each variable.

that what was defined as poor metabolic status in wk 2 is different from what was defined as poor metabolic status in wk 7. However, comparing clusters 1 and 3 over lactation weeks shows that the differences over lactation weeks among clusters are marginal, indicating that the cows defined as having a poor metabolic status have a similar metabolic profile over the first 7 lactation weeks in the current study.

In a bi-plot of PCA, the opposing loadings of 5 plasma metabolites and metabolic hormones could indicate how these values contribute to the separation of dairy cows into good, average, or poor metabolic status. The negative contribution of plasma BHB and FFA to the first PC means that high BHB and FFA are related to poor metabolic status in cluster analysis. Concentration of FFA is an indicator of fat mobilization during poor metabolic status, whereas plasma BHB is an indicator of

fat metabolism and incomplete oxidation of fat (Grummer, 1993). In our study, cows with poor metabolic status had the lowest concentration of glucose, insulin, and IGF-1 among 3 clusters. Glucose in early lactation is essential not only for lactose production but also for metabolizing fatty acids and making energy available to the body in the form of ATP (van Knegsel et al., 2005). Low insulin reduces glucose uptake by insulin-responsive peripheral tissues (adipose and muscle) and facilitates greater uptake of glucose by the mammary gland (Bauman and Elliot, 1983). Low concentration of IGF-1 and insulin is related to the negative energy balance status of cows (Lucy et al., 1992). Cows with the greatest concentration of insulin and IGF-1 were defined as having a good metabolic status, which fits with the observation that great concentrations of insulin and IGF-1 were found in cows with positive energy balance

(Lucy et al., 1992; McGuire et al., 1995). Associated with increasing plasma glucose and insulin availability, increased IGF-1 can be hypothesized to have anabolic effects on carbohydrate metabolism (Harding et al., 1994; Rigout et al., 2002). Cows with good metabolic status had greater IGF-1 (Table 2), lower BW, and lower average parity (Supplemental Table S3, <https://doi.org/10.3168/jds.2018-15791>). In mammals, IGF-1 promotes growth and aging (Gems and Partridge, 2001; Sonntag et al., 2005), which could indicate that cows in good metabolic status have a more anabolic status. Therefore, cows in good metabolic status have low BW, milk yield, protein yield, and fat yield (Supplemental Table S3, <https://doi.org/10.3168/jds.2018-15791>). The low average BW and low average parity in this cluster suggest that there might be a greater proportion of young cows that still have some priority for growth in this cluster, which might explain the greater plasma IGF-1 concentration for these cows.

In the current study, machine learning algorithms were used to predict cows in good or average metabolic status but not cows in poor metabolic status. This approach was chosen due to a limited number of animals in the cluster with a poor metabolic status to improve the predictive performance of the algorithms. For cow health management, however, prediction of cows in poor metabolic status is very informative. Therefore, evaluation of machine learning algorithms to predict cows in poor, average, or good metabolic status is included in Supplemental Table S2 (<https://doi.org/10.3168/jds.2018-15791>). Identification of metabolic status could facilitate cow health management by fine-tuning dietary and management strategies for individual dairy cows in a precision farming system (as reviewed by Rutten et al., 2013). On the one hand, cows in poor metabolic status could be supplied with a diet with greater energy content or assigned to a 30- or 0-d DP to improve metabolic status in the next lactation. On the other hand, cows in good metabolic status might deserve extra attention to prevent metabolic diseases in the next lactation caused by body fat accumulation in the current lactation (Grummer, 1993). First, limiting energy supply could result in reduced body fat storage and lower BCS (Dewhurst et al., 2000). Second, cows with good metabolic status are candidates for a 30- or 60-d DP to reduce energy balance and maximize milk yield in the next lactation (Chen et al., 2016).

Predicting Metabolic Status with Machine Learning Algorithms

In this study, *k*-means was an unsupervised learning method in cluster analysis, whereas K-Nearest Neighbor

was a supervised learning method to predict metabolic status. Although both algorithms used Euclidean distance as kernel function, K-Nearest Neighbor could limitedly predict metabolic status with on-farm cow data. It is difficult to consider a specific linear function with conventional statistical methods due to its limitations when using correlated variables. In this context, analyzing data through machine learning algorithms seems promising to manage large data sets with on-farm cow data with a lot of correlations among features (Caraviello et al., 2006).

The superior performance of Random Forest and Bootstrap Aggregation is in line with a study that predicts insemination outcomes in cows (Shahinfar et al., 2014), which could be explained by the power of ensemble methods to generate high-performance classifiers by collecting individual trained classifiers. Although Random Forest performed better than the other algorithms, even to predict cows with 3 metabolic statuses in our study (Supplemental Table S2, <https://doi.org/10.3168/jds.2018-15791>), it does not mean that Random Forest will always be the best algorithm in practice. The performance of all algorithms (e.g., Random Forest) depends on used features and parameters. A multiparallel comparison among algorithms, therefore, is necessary to evaluate algorithms in a specific case.

Other machine learning algorithms had a relatively higher error rate than Random Forest and Bootstrap Aggregation. Among all algorithms, ANN is frequently used, for example, to predict milk yield (Grzesiak et al., 2006; Gianola et al., 2011) and classify mastitis cases (Cavero et al., 2008; Sun et al., 2010). In our study, however, the error rate of ANN (21.3–39.2%; Table 3) in prediction of metabolic status is high compared with other studied algorithms. The reasons for difference in fit of ANN among studies are unknown.

All algorithms had a better prediction of metabolic status of cows in lactation wk 2 and 3 compared with prediction in wk 1 and 4 to 7, which could be explained by the dramatic changes of metabolic status at the beginning of lactation (Drackley, 1999). Dramatic changes of metabolic status in cows with low adaptive capacity in early lactation could benefit the prediction of metabolic status by machine learning algorithm in the current study. In addition, major incorrectly predicted cases in Random Forest were presented in the border area between good and average metabolic status of cows in the PCA plot (Figure 1). This result indicates that on-farm cow data are limited to predict metabolic status of cows in the border area between clusters, which could be explained by some features that are not different between cows in good or average metabolic

status, such as BW, fat percentage, lactose percentage, and FPCM (Supplemental Table S3, <https://doi.org/10.3168/jds.2018-15791>).

The performance of algorithms with different sensitivity, specificity, PPV, and NPV determines application in practice. The relative higher sensitivity and NPV value of Random Forest could be helpful to detect cows with surplus energy balance (also referred to good metabolic status). It would be expected to further study the reason why machine learning algorithms perform differently in sensitivity, specificity, PPV, and NPV and improve the performance of applied algorithms in practice (e.g., how the sensitivity of prediction of metabolic status could be improved using dietary component data). Moreover, several other aspects can be hypothesized to facilitate further fine-tuning of machine learning algorithms to predict metabolic status. First, larger data sets and more comprehensive on-farm cow data or on-farm non-cow data (e.g., data sets including more cows in poor metabolic status) would allow more precise prediction of cows in poor metabolic status, and weather data could be used to predict energy intake of dairy cows (West et al., 2003), which could be related to metabolic status in early lactation. Second, in the current study, features were included as the weekly average or standard deviation per week for milk yield. Also, other on-farm cow data can be hypothesized to contribute to a model to estimate metabolic status, such as minimum or maximum milk yield, feeding activity, or lying time per day (van Hoeij et al., 2019). Third, models could be further optimized using not only data per lactation week but also data of all lactation weeks at once. This would imply that the random effect of individual cows in consecutive lactation weeks needs to be considered, which would request more complicated algorithms, which are, to our knowledge, not standard procedure. Fourth, machine learning algorithms can be used to predict not only metabolic status, as in Tremblay et al. (2018) or the current study, but specific diseases and disorders associated with metabolic status, such as fatty liver or subclinical ketosis in dairy cows (Pralle et al., 2018).

Important Features

In the current study, Random Forest had the relative lower error rate but also had the highest sensitivity and NPV to predict 362 dairy cows with good metabolic status. In lactation wk 1, 2, and 3, milk yield, fat yield, and protein percentage played important roles in predicting cows in good or average metabolic status in Random Forest, which could be explained by relation between

milk production and body fat mobilization. Increased fat yield in early lactation originates partly from mobilized body fat; this process is related to elevated plasma concentration of FFA and BHB (Grummer, 1993). Therefore, milk fat and fat:protein ratio were used to estimate energy balance of cows at the herd level (Friggens et al., 2007; Butchereit et al., 2010). In the current study, in lactation wk 4 and 5, protein percentage and lactose yield were the top 2 important features in Random Forest. It can be speculated that milk protein and lactose are important to assign cows to good or average metabolic status. Cows in average metabolic status had greater protein and lactose production than cows in good and poor metabolic status (Supplemental Table S3, <https://doi.org/10.3168/jds.2018-15791>). In lactation wk 6 and 7, fat-related features were not as important as milk yield, milk protein percentage, and lactose yield. In lactation wk 6 and 7, cows in average metabolic status had the same fat yield as cows in poor metabolic status (Supplemental Table S3, <https://doi.org/10.3168/jds.2018-15791>), which could be explained by the recovery to positive energy balance status after 41.5 DIM postpartum (de Vries and Veerkamp, 2000). Fine-tuning the model by adjusting the contribution of milk yield, fat yield, protein percentage, and lactose yield in each lactation week to the model could improve predictive performance in further studies.

CONCLUSIONS

Through lactation wk 1 to 7, dairy cows were clustered by poor, average, or good metabolic status based on 5 plasma metabolites and metabolic hormones. On-farm cow data predicted cows in good or average metabolic status in early lactation using 8 machine learning algorithms. Error rate of those algorithms ranged from 12.4 to 49.8%. Of all algorithms, Random Forest and SVM had stable and best performance to predict metabolic status of dairy cows. Milk yield, fat yield, protein percentage, and lactose yield had important roles in Random Forest; however, their rank of importance differed across lactation weeks.

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APPENDIX

Machine Learning Algorithms

Decision Tree is a decision support model with a tree-like graph presenting decisions and their possible consequences on each node. As one widely used algorithm in Decision Tree (Wu et al., 2008), the C4.5 algorithm constructs subtrees (lower levels of the tree) through recursive partitioning of each sample, choosing the largest information gain ratios in earlier iterations (higher levels of the tree) to divide each instance (Quinlan, 1996). If all remaining instances belong to the same class, then the C4.5 algorithm stops building subtrees. The information gain (IG) is defined as

$$IG(D, a) = Entropy(D) - \sum_{v=1}^V \frac{|D^v|}{|D|} Entropy(D^v),$$

where

$$Entropy(D) = - \sum_{k=1}^{|y|} p_k \log_2 p_k,$$

where D is a sample set with k class; p_k is the proportion of example in class k ; discrete attribute a has v possible values $a_1, a_2, \dots, a_v$; and D^v is a set including all samples with the same v in attribute a . There is no setting to parameters “weights” and “subset”; “parms” is “1” (default value); and “method” is “class.”

Naïve Bayes is a classification technique based on Bayes’ Theorem, where all features are supposed to be independent (Domingos and Pazzani, 1997). Although this assumption rarely holds true in real-world applications, Naïve Bayes still performs well in many cases (Domingos and Pazzani, 1997; Ng and Jordan, 2001), based on Maximum a Posteriori principle:

$$\hat{y} = \arg \max_{c \in y} P(c) \prod_{i=1}^d P(x_i | c), \quad [A1]$$

where y is a set of class c ; $P(c)$ is prior probability; $P(x_i | c)$ is class-conditional probability of x in feature i as class c ; and d is the sum of features. There was no setting to parameters “subset,” “na.action,” “sampsize,” and “threshold,” and “laplace” is “0” (default).

Bayesian Network is a probabilistic graphical model developed from Naïve Bayes. In graphical models of Bayesian Network, each node represents a random variable, and the edge between the nodes represents probabilistic dependencies among corresponding random variables. Bayesian Network therefore enables an effective representation and computation of joint probability distribution over a set of random variables (Pearl, 2014). There is no setting to the parameters “dist,” “ordinal,” and “keep.fitted,” and “method” is “bayes-lw.”

In Support Vector Machine, each observation is plotted as a point in n -dimensional space (n is the number of features in the data set) with the value in a particular coordinate of each feature. Then, separating the classes with a hyperplane maximizes the margin between them. The sample point closest to the hyperplane is termed “support vector” (Hsu et al., 2003). There is no setting to the parameters “subset,” “startweights,” “lifesign,” and “maxnodes”; “tolerance” is “0.001” (default); “epsilon” is “0.1” (default); “cachesize” is “40” (default); “degree” is “3” (default); “coef0” is “0” (default); “shrinking” is “TRUE” (default); and “fitted” is “TRUE” (default).

Artificial Neural Networks consist of interconnected processing units (nodes). Each node consists of a summing part receiving N input values, weights, and bias to compute a weighted sum. The output part produces a signal from the weighted sum (Yegnanarayana, 2009). Processing units process data and learn to recognize linear or nonlinear patterns through iterative learning. There is no setting to the parameters “err.fct,” “act.fct,” and “exclude”; “likelihood” is “TRUE” (default); “hidden” is “c(5)”; “stepmax” is “1e6”; “threshold” is “0.1”; and “linear.output” is “FALSE.”

Bootstrap Aggregation is a method to generate multiple classifiers to get an aggregated predictor (Breiman, 1996a). Predictors are constructed using bootstrap samples in training data sets and then aggregated to form a Bootstrap predictor. Each bootstrap step leaves out about 37% of samples as out-of-bag samples for testing (Breiman, 1996b). There is no setting to the parameters “nbagg,” “na.action,” and “coob,” and “method” is “standard.”

Random Forest is based on Decision Tree algorithm with a group of tree-structured classification models. In Random Forest, many classifiers are trained using m bootstrap samples from the training data set, which is in line with Bootstrap Aggregation. A subset of features is randomly selected to generate each classifier in Random Forest, whereas Bootstrap Aggregation uses all features (Breiman, 2001; Liaw and Wiener, 2002).

There is no setting to the parameters “subset,” “na.action,” “sampsize,” and “maxnodes”; “mtry” is “3” (default); “nodesize” is “1” (default), “ntree” is “500,” and “proximity” is “TRUE.”

K-Nearest Neighbor is a widely used nonparametric classifier. It classes tested samples through the distance function built with the training data set and prerequisite parameter K . Euclidean distance is the most widely used distance function for continuous variables in KNN (Hu et al., 2016). Optimal K is a prerequisite determined by cross-validation. It is a type of instance-based algorithm, where the function is only approximated locally and all computation is deferred until classification; therefore, off-line training is not needed (Tan, 2006). There is no setting to the parameters “prob” and “l,” and “k” is “10.”