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Artificial neural network models of the rumen fermentation pattern in dairy cattle

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ABSTRACT

The objectives of this study were: (1) to predict the rumen fermentation pattern from milk fatty acids using a machine learning technique, i.e. artificial neural networks (ANN) combined with feature selection and (2) to compare the prediction accuracy of the resulting model to that of a statistical multi-linear regression model, based on odd and branched chain milk fatty acids. Data were collected from 10 experiments with rumen fistulated dairy cows, resulting in a dataset of 138 observations. Feature selection was based on correlation and principal component analysis, and background physiological knowledge. Different ANN architectures and training algorithms were assessed. The evaluation of the model performance, based on the test dataset, showed a root mean square prediction error, expressed relative to the observed mean, of 2.65%, 7.67% and 7.61% of the observed mean for acetate, propionate and butyrate, respectively. Compared to a multi-linear regression model, the ANN revealed not to perform significantly better. However, the results confirm that milk fatty acids have great potential to predict molar proportions of individual volatile fatty acids in the rumen.

1. Introduction

Volatile fatty acids (VFA), produced by rumen microbes, are the major source of glucogenic and lipogenic nutrients to the ruminant. The relative proportions of the individual VFA, mainly acetate, propionate and butyrate, vary widely according to the animal's diet and influence the energy utilisation and the amount and composition of the milk produced (Sutton, 1985; Mc Donald et al., 1995). Prediction of the VFA proportions is therefore an important means of evaluating diets for milk production. Hence, much effort has already been done for the prediction of the VFA in the rumen. Mechanistic models (Baldwin et al., 1987; Dijkstra et al., 1992), based on feed characteristics and rumen conditions, were used, but the prediction accuracy of molar proportions VFA is low (Bannink et al., 1997). Friggens et al. (1998) used empirical regression equations, based on feed composition, but these regression models have not been tested on independent validation data. Also the gas production technique, another technique based on feed characteristics, was used to predict VFA proportions (Brown et al., 2002 and Rymer and Givens, 2002). However, the accuracy of these prediction models is still variable. This might be related to the fact that differences in the rumen fermentation pattern and bacterial population are not only affected by dietary composition but are also influenced by factors such as animal variability and feeding management (AFRC, 1998; Weimer et al., 1999; Vlaeminck et al., 2004). Hence, our laboratory approach is to use profiles of particular milk fatty acids (MFA) to improve rumen VFA predictions. Recently, Vlaeminck et al. (2006a) developed a multi-linear regression model based

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on odd and branched chain fatty acids (OBCFA) in milk for the prediction of VFA proportions, which revealed to perform better (root mean square prediction error <10% of the observed mean) compared to the existing models. The major source of these fatty acids in milk is from rumen bacterial origin rather than from animal synthesis (Vlaeminck et al., 2006b). Therefore, profiles of these MFA might be used as a tool to assess rumen function in terms of microbial populations, substrates and their interactions (Cabrita et al., 2003; Vlaeminck et al., 2006b). Prediction models, based on MFA, include animal variability because the MFA are measured for each individual cow and therefore can give information about individual rumen conditions.

Machine learning techniques, such as artificial neural networks (ANN), might further improve rumen VFA predictive models based on MFA through their capability of handling non-linear and complex data, even if these data are noisy and imprecise (Lek and Guégan, 1999). Moreover, ANNs have been shown to yield universal and highly flexible function approximates for any data (Lek and Guégan, 1999). Machine learning techniques are therefore increasingly used for classification and prediction purposes in agriculture (Salehi et al., 1998; Heald et al., 2000; Stefanon et al., 2001; Pietersma et al., 2003). This technique might be interesting when predictive MFA respond to rumen conditions in a non-linear way. C18-hydrogenation intermediates, especially *trans*-10 C18:1, are an example of a well-known non-linear response to rumen circumstances. *Trans*-10 C18:1 reflects more acidogenic rumen conditions, although the relationship with rumen pH is highly non-linear (Loor et al., 2005; Kalscheur et al., 1997). The use of ANN as modelling technique is therefore investigated in order to allow the inclusion of these non-linear relationships between MFA and rumen proportions of VFA.

The aim of this paper is to evaluate the use of ANN for the prediction of rumen proportions of VFA using MFA profiles. The prediction accuracy of the ANN is compared with that of a statistical multi-linear regression model based on milk OBCFA, using the same independent literature data.

2. Materials and methods

2.1. Data description

The current study combined data from 10 different experiments resulting in a dataset of 138 individual observations (Table 1) (Vlaeminck et al., 2006a).

2.1.1. *Experiment 1 (n = 17)*

Experimental design and diets were previously described by Dewhurst et al. (2003). This experiment was according to a four-period incomplete changeover design, in which six cows in the beginning of the lactation (76 ± 36 DIM) were used to test six dietary treatments. Each experimental treatment lasted for 3 weeks, of which the first 2 weeks were for adaptation. Each cow was offered four different diets. The cows received 8 kg/day of concentrate in three portions. Cows had ad libitum access to one of six silages: grass, red clover, white clover, alfalfa and 50/50 (DM basis) mixtures of grass and red clover and grass and white clover.

2.1.2. *Experiment 2 (n = 16)*

Experimental design and diets were described by Moorby et al. (2006) and Vlaeminck et al. (2006c). This experiment was according to a 4 × 4 Latin square design, in which four dairy cows (90 ± 34 DIM at the beginning of the experiment) were offered diets varying in forage to concentrate ratio. Each experimental period lasted for 4 weeks of which the first 2 weeks were for adaptation. Dietary treatments were based on ad libitum access to ryegrass silage and a dairy concentrate with forage to concentrate ratios of 80:20, 65:35, 50:50, 35:65 (DM basis). Fresh forage was distributed daily at 09.00 h whereas concentrates were distributed twice daily in equal portions at milking (Moorby et al., 2006; Vlaeminck et al., 2006c).

2.1.3. *Experiment 3 (n = 16)*

Experimental design and diets were described by Hindle et al. (2005). This experiment was a 4 × 4 Latin square design, in which four multi-cannulated dairy cows (80 ± 18 DIM at the beginning of the experiment) received a control diet, consisting of grass silage (43% of DM), ensiled sugar beet pulp (11% of DM) and a concentrate mixture with 70% dried sugar beet pulp. Dried sugar beet pulp of the concentrate was replaced either by native potato starch, cornmeal, or wheat meal in each of the three experimental diets. Each experimental period lasted for 4 weeks of which the first 2 weeks were for adaptation.

2.1.4. *Experiment 4 (n = 16)*

Experimental procedures are described in detail by Bruinenberg et al. (2004). Four rumen-cannulated multiparous Holstein cows (249 ± 76 DIM at the beginning of the experiment) were assigned to a 4 × 4 Latin square experiment. The experimental period lasted for 3 weeks, of which the first 2 weeks were for adaptation. The four dietary treatments consisted of different combinations of three grassland silages.

2.1.5. *Experiments 5 and 6 (n = 49)*

These experiments were both 5 × 5 Latin squares, with five dairy cows in early lactation at the beginning of experiment 5 (45 ± 14 DIM) and five dairy cows in late lactation at the beginning of experiment 6 (236 ± 14 DIM). The cows were offered diets varying in source of forage and concentrate (55/45 DM basis). Dietary treatments were based on ad libitum access to one of the five TMR: (1) a mixture (50/50, DM basis) of ryegrass silage and corn silage as forage and a mixture (50/50, DM basis) of two concentrates either rich in structural or in non-structural carbohydrates; (2) ryegrass silage as forage and the concentrate mixture (50/50, DM basis) as in diet 1; (3) corn silage as forage and the concentrate mixture (50/50, DM basis) as in diet 1; (4) a mixture (50/50, DM basis) of ryegrass silage and corn silage as forage and a concentrate rich in structural carbohydrates; (5) a mixture (50/50, DM basis) of ryegrass silage and corn silage as forage and a concentrate rich in non-structural carbohydrates. Each experimental period lasted for 3 weeks, of which the first 2 weeks were for adaptation.

2.1.6. *Experiments 7 and 8 (n = 17)*

These two experiments were both 3 × 3 Latin squares. Three dairy cows (294 ± 148 DIM at the beginning of the experiment) were offered diets with corn and grass silage as forage with a standard dairy concentrate. Corn silage was taken from two

Table 1 – Overview of the 10 experiments used for the development of artificial neural networks to predict rumen volatile fatty acid proportions

Experiment	Data	Cows	Lactation stage (days in milk)	Experimental period (days)	Roughage	Concentrate (kg/day)	Experimental design	Test objectives	Description (design + diet)
1	17	6	76 ± 36	21	Grass, clover, alfalfa silage or mixed silages	8	4 × 6	Evaluation of feed intake, milk production, N utilisation and fatty acid composition of milk fat with diets based on legumes silages	Dewhurst et al. (2003)
2	16	4	90 ± 34	28	Grass silage	F:C ratios (80/20,65/35, 50/50, 35/65)	4 × 4	Relationship between forage–concentrate ratio and feed intake, supply and utilisation of nutrients in rumen	Moorby et al. (2006)
3	16	4	80 ± 18	28	Grass silage	17	4 × 4	Relate site and extent of degradation of three starch sources, determined in vivo and compared with nylon bag and gas production technique	Hindle et al. (2005)
4	16	4	249 ± 76	21	Grass silage	4.5	4 × 4	Assessment of effect of grassland management on ruminal digestion	Bruinenberg et al. (2004)
5 + 6	49	5	45 ± 14 (exp. 5) 236 ± 14 (exp. 6)	21	Grass + maize silage	TMR (50/50)	5 × 5	Effect of varying forage (maize vs. grass silage) and concentrate (starch vs. fiber rich) source on diurnal intake pattern and milk fatty acid composition	Abrahams et al. (unpublished results)
7 + 8	17	3	294 ± 148	13	Grass + maize silage	5.1	3 × 3	Effect of starch content of corn silage on rumen fermentation pattern and milk production characteristics	De Brabander et al. (2004)
9 + 10	7	2	278 ± 108	13	Grass + maize silage	5.1	2 × 2	Effect of starch degradability of corn silage on rumen fermentation pattern and milk production characteristics	De Brabander et al. (2005)

different varieties varying in starch content. Dietary treatments were based on ad libitum access of forage and 5.1 kg/day of the standard dairy concentrate. Each experimental period lasted for 13 days, of which the first 10 days were for adaptation.

2.1.7. *Experiments 9 and 10 (n = 7)*

These two experiments were both 2×2 Latin squares. Two dairy cows (278 ± 108 DIM at the beginning of the experiment) were offered diets with corn and grass silage as forage with a standard dairy concentrate. Corn silage was from two different varieties, inducing variation in rumen bypass starch content. Dietary treatments were based on ad libitum access of the forage and 5.1 kg/day of a standard dairy concentrate. Each experimental period lasted for 13 days of which the first 10 days were for adaptation.

For more detailed information we refer to Vlaeminck et al. (2006a) and the references, included in Table 1.

2.2. *Analysis*

In all the experiments, milk samples were extracted, methylated and analysed by gas liquid chromatography as described by Vlaeminck et al. (2005). Milk fatty acids were expressed as g/100 g fatty acids. Mean 24-h molar proportions of acetate, propionate and butyrate [mmol/mol (acetate + propionate + butyrate)] were calculated from the results of individually analysed rumen samples during the day.

Concentrations of individual MFA (C4:0, C6:0, C8:0, C10:0, C12:0, *iso* C13:0, *anteiso* C13:0, *iso* C14:0, C14:0, *iso* C15:0, *anteiso* C15:0, *cis*-C14:1, C15:0, *iso* C16:0, C16:0, *trans*-C16:1, *iso* C17:0, *cis*-C14:1, *anteiso* C17:0, C17:0, *cis*-C17:1, C18:0, *trans*-6 C18:1, *trans*-9 C18:1, *trans*-10 C18:1, *trans*-11 C18:1, *cis*-9 C18:1, *cis*-11 C18:1, C18:2, *trans*-11 *cis*-15 C18:2, *cis*-9 *trans*-11 C18:2, *trans*-10 *cis*-12 C18:2, C18:3, C20:0, C20:1) and rumen proportions of VFA (acetate, propionate and butyrate) were used to construct the model. However, for the short-chain fatty acids (C4:0–C10:0), which are synthesised de novo in the udder, the sum has been used instead of the individual concentrations. Similarly, the sum of C17:0 and *cis*-9 C17:1 has been considered, since *cis*-9 C17:1 is a desaturation product of C17:0 produced in the mammary gland (Fievez et al., 2003).

2.3. *Data preprocessing*

2.3.1. *Normalisation of input and output data*

Because the different variables span different ranges and in order to ensure that all the variables receive equal attention during the training process, it is recommended to rescale the data (Maier and Dandy, 2000).

Data were normalised into the interval [−1, 1] using the following expression:

$$Y = 2\frac{X - X_{\min}}{X_{\max} - X_{\min}} - 1 \quad (1)$$

with X and Y the original and normalised input or output data, and $X_{\min}$ and $X_{\max}$ the minimum and maximum values of the input and output data.

Also the output data were rescaled into the interval −1 and 1 to allow for the application of the transfer function (tangential sigmoid function) in the ANN.

2.3.2. *Selection of input variables*

The presence of too many input variables can cause poor generalisation and reduces the predictive ability of ANN when the ANN not only models the true predictors, but includes irrelevant and redundant variables in the model (Piramuthu, 2004). Feature selection is therefore an essential step during ANN development. Walczak and Cerpa (1999) mentioned heuristic methods to determine the optimal set of input variables prior to model development. First, there is standard knowledge acquisition, which involves consultation with multiple domain experts. Further, ANN input variables should not be correlated, because correlated variables degrade ANN performance by interacting with each other to produce a biased effect. A filter to help identify noise variables is to calculate the correlation of pairs of variables (Pearson correlation matrix). If two variables are correlated (absolute value Pearson correlation coefficient of 0.20 or higher), one of these two variables may be removed from the set of variables without adversely affecting the ANN performance. Additionally, loading plots of principal component analysis were used to identify correlated variables.

During ANN development, the contribution of variables in ANN can further be calculated by different methods, where the connection weights approach provides the best overall methodology to accurately quantify variable importance (Olden et al., 2004). This feature selection method, during model development, calculates the product of the raw input-hidden and hidden-output connection weights (Fig. 1) between each input neuron and output neuron and sums the products across all hidden neurons. Input variables, receiving larger connection weights, represent greater intensities of signal transfer and are therefore more important in the prediction process (Olden and Jackson, 2002; Olden et al., 2004).

2.3.3. *Partitioning of data*

The data from the 10 experiments were grouped into one dataset. In contrast to Vlaeminck et al. (2006a), the factor 'experiment' was not considered during the development of the ANN. The former used 'experimental trial' as a random factor in the multi-linear regression models for the prediction of rumen proportions of VFA. No information about the origin of the data is included in the ANN models. In this way, an experiment-independent model is developed. The performance of an ANN model without experiment-related information can therefore be compared to that of a multi-regression model (Vlaeminck et al., 2006a), in which experiment was included as a random factor in order to improve the estimation of the regression parameters. The dataset (138 observations) was split into training (92 observations), validation (11 observations) and test (35 observations) data. Data were sorted by acetate proportions, which facilitated the selection of the validation and test data from the dataset in order to ensure a similar distribution of the input and output variables in all three datasets (Table 2). The range of input and output variables is wide, what is explained by the inclusion of different animals, diets and environments in the

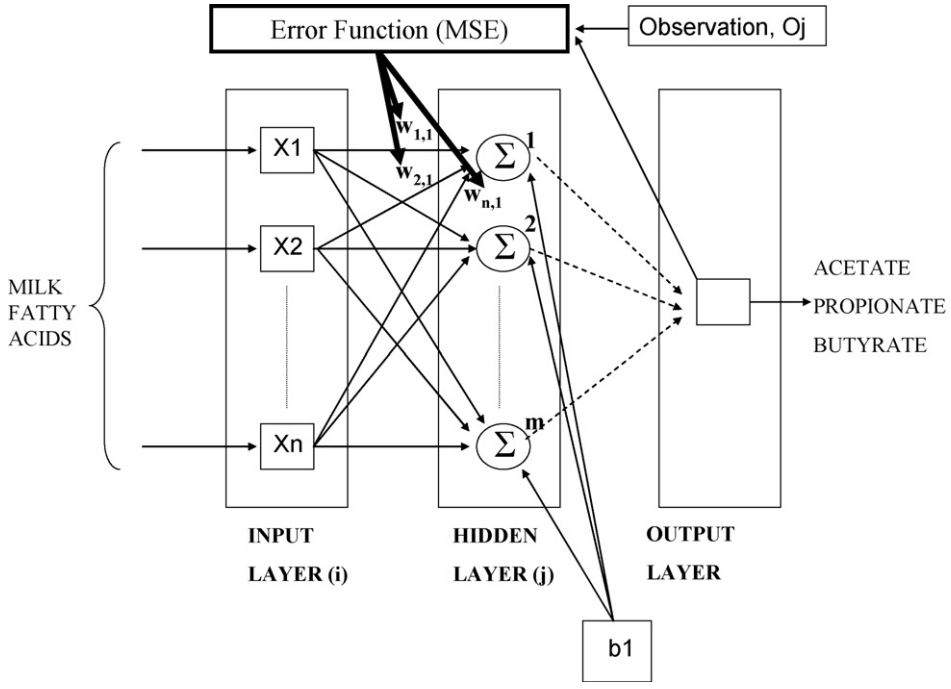

Fig. 1 – Illustration of a backpropagation neural network with one input layer, one hidden layer and *m* neurons in the hidden layer. The milk fatty acids (input vectors) are connected by weights (*w*) to the neurons in the hidden layer. This signal is transferred (⋯▶) by a transfer function, resulting in an output value (acetate, propionate or butyrate). The error function is calculated as the mean square prediction error between the observed and the predicted values. The weights and biases are adapted (⟶) in order to decrease the error function.

dataset and which is essential to ensure robust model development applicable under different feedstuffs and animals. The training data are used to train the ANN. A significant problem with ANN training is their tendency to overtrain, which means that the network learns the noise within the training data and loses the ability to accurately predict the properties of records excluded from the training data (Plumb et al., 2005). This can be prevented by the 'early stopping' technique which measures the prediction error on the validation data during the training process. The validation error decreases normally during the initial phase of training, as does the training set error. When the ANN starts to overfit the data, the error on the validation set will typically begin to rise. When the validation error increases for a specified number of iterations, the training process is stopped, and the weights and biases at the minimum of the validation error are returned (Demuth et al., 2005). In the testing phase, input vectors are given to the network, and the predicted output values are compared with the observed values.

2.4. *Artificial neural networks*

In the present work, different ANN were developed using the Matlab® Neural Network Toolbox. The backpropagation network (BPN), which is also referred to as the multilayer perceptron, is currently the most general-purpose, and commonly used neural network paradigm. The BPN (Fig. 1) is a layered, feed-forward network, comprised of one input layer, one or more hidden layers and one output layer (Skapura, 1996). The ANN receives information in the input layer by input vectors, passes this information through neurons and finally gives a certain output value in the output layer. The input of one neuron in the hidden layer is the sum of all the input signals, multiplied with a connection weight and a bias term. This input signal is converted into an output signal by a transfer function, which can be sigmoid, tangential or linear. In this study, a tangential transfer function is used in the hidden as well as in the output layer. All the neurons from one layer are connected to all the neurons of the following layer, but there are no feed-back connections, or lateral connections within any layer. The BPN is based on a supervised procedure, i.e. the network constructs a model based on examples of data with known outputs (Lek and Guégan, 1999; Plumb et al., 2005).

During the training process, the training data are presented to the network multiple times, which results in an iterative training process where the weights and biases are updated in order to decrease the error function between the actual and the predicted training data (Skapura, 1996; Lek and Guégan, 1999). A large number of training algorithms for feed-forward neural networks were proposed in literature. Commonly used algorithms for training ANN are the Levenberg–Marquardt algorithm, the gradient descent algorithm and the scaled conjugate gradient algorithm (Demuth et al., 2005) and are therefore compared in this study. The gradient descent algorithm updates the network weights and biases in the direction in which the performance function decreases most rapidly, i.e. the negative of the gradient. Minimization by this algorithm is based on a linear approximation of the Taylor expansion of the error function. With standard gradient descent algorithms, the learning rate, which controls the

Table 2 – Statistics (average, standard deviation, minimum and maximum) of the experimental data for the development of the artificial neural network after the first feature selection for training ($n = 92$), validation ($n = 11$) and test data ($n = 35$)

	Average			Standard deviation			Minimum			Maximum		
	Training	Validation	Test	Training	Validation	Test	Training	Validation	Test	Training	Validation	Test
Milk fatty acids (g/100 g of fatty acids)												
C4:0 + C6:0 + C8:0 + C10:0	10.4	10.3	10.5	1.26	1.22	1.58	5.36	8.71	6.41	13.3	12.5	13.7
Anteiso C13:0	0.087	0.086	0.087	0.027	0.029	0.032	0.020	0.043	0.021	0.161	0.147	0.184
Iso C14:0	0.084	0.089	0.085	0.020	0.032	0.021	0.044	0.043	0.031	0.144	0.154	0.129
Iso C15:0	0.220	0.210	0.220	0.041	0.037	0.044	0.117	0.116	0.116	0.325	0.260	0.316
Anteiso C15:0	0.474	0.471	0.472	0.069	0.072	0.062	0.309	0.375	0.350	0.701	0.611	0.654
C15:0	1.08	1.12	1.07	0.166	0.165	0.151	0.823	0.845	0.700	1.56	1.39	1.36
C16:0	31.0	31.7	31.0	3.69	4.63	4.14	22.3	25.6	21.7	40.4	42.4	42.4
Iso C17:0	0.190	0.185	0.191	0.043	0.031	0.040	0.111	0.138	0.120	0.360	0.244	0.294
Anteiso C17:0	0.539	0.536	0.525	0.104	0.081	0.096	0.357	0.353	0.397	0.833	0.633	0.760
C17:0 + *cis*-9 C17:1	0.694	0.740	0.692	0.106	0.155	0.089	0.507	0.557	0.549	1.13	1.09	0.985
Trans-10 C18:1	0.466	0.889	0.537	0.624	1.66	0.914	0.080	0.100	0.077	4.18	5.82	4.59
Cis-9, *trans*-11 C18:2	0.540	0.531	0.520	0.205	0.212	0.240	0.185	0.131	0.169	1.25	0.859	1.06
Trans-10, *cis*-12 C18:2	0.019	0.028	0.020	0.017	0.030	0.027	0.005	0.006	0.007	0.114	0.112	0.159
Rumen fermentation pattern (mmol/mol)												
Acetate	660	655	658	33.5	43.9	38.1	567	559	548	724	719	724
Propionate	210	206	209	27.3	37.7	33.5	158	169	162	308	303	322
Butyrate	132	127	133	17.5	14.5	16.7	98.3	106	102	183	149	184

update step size, is held constant throughout training, so the performance will be very sensitive to the setting of this learning rate. The performance of the gradient descent algorithm can be improved if the learning rate varies along the course of training by making it responsive to the complexity of the local error surface (Møller, 1993). A momentum term was added to the training algorithm to help the search escape local minima and reduce the likelihood of search instability (Basheer and Hajmeer, 2000). Therefore, in this study, the gradient descent algorithm with a variable learning rate and momentum term is used.

While the gradient descent technique is a steepest descent algorithm, the Levenberg–Marquardt algorithm is an approximation to Newton's method. It is designed to approach second-order training speed with a simplified form of the Hessian matrix (second derivatives).

Møller (1993) developed the scaled conjugate gradient algorithm which is a combination of the gradient descent and the Levenberg–Marquardt algorithm. In this algorithm a search is performed along conjugate directions, which generally leads to faster convergence than steepest gradient descent directions. In the gradient descent algorithm, a learning rate is used to determine the length of the weight update (step size). In the scaled conjugate gradient algorithm, the step size is adjusted at each iteration (Demuth et al., 2005). This method chooses the search direction and the step size from the second-order approximation of the error function (Møller, 1993).

The optimal ANN geometry is highly problem-dependent and should be designed by trial and error. The number of hidden layers is set to one, because an ANN with one hidden layer can approximate any function as long as sufficient neurons are used. The determination of the appropriate number of hidden nodes in the hidden layer is a critical task in an ANN design. When an ANN has too many hidden neurons, it will model the noise in the data, due to overparameterization leading to poor generalisation capacity (Basheer and Hajmeer, 2000). The number of neurons is chosen by trial and error.

2.5. *Comparison of predicted and observed values*

The accuracy of the ANN is evaluated on the independent test data ($n = 35$) collected from the dataset (Table 1) used for model development. Furthermore, independent literature data ($n = 14$) from experiments, described by Shingfield et al. (2003, 2005) and Loor et al. (2005) are used to validate the ANN.

The results of the ANN were validated using the mean square prediction error (MSPE) (Bibby and Toutenberg, 1977):

$$\mathrm{MSPE} = \frac{1}{n}\sum_{i=1}^{n}(A_i - P_i)^2 \quad (2)$$

where n is the number of observations, A_i and P_i are the observed and predicted values, respectively. The square root of MSPE (RMSE) is expressed in the same units as the observed values and a comparison of the RMSE as percentage of the observed mean gives an indication of the overall error of prediction. The MSPE can further be regarded as the sum of three components:

$$\mathrm{MSPE} = (\bar{A} - \bar{P})^2 + S_P(1 - b^2) + S_A(1 - r^2) \quad (3)$$

where S_A^2 and S_P^2 are the variances of the actual and the predicted values, $\bar{A}$ and $\bar{P}$ the means of the actual and the predicted values, b the slope of the regression of A on P and r^2 is the correlation coefficient between actual and predicted values. The three components are due to mean bias $[(\bar{A} - \bar{P})^2]$, line bias $[S_P(1 - b^2]$ and random variation around the regression line $[S_A(1 - r^2)]$.

The ANN is also evaluated by residual plots of the independent data. The residuals are regressed against predicted values to assess the model's mean and linear biases. A positive or negative slope of the residuals on the predicted values is a test of biased prediction (St-Pierre, 2003).

3. Results and discussion

3.1. *Selection of input variables*

The correlation matrix and principal component loading plot were assessed including all MFA. From these results and standard knowledge acquisition, a first selection of possible input variables has been made (Table 2). The standard knowledge acquisition was based on background physiological knowledge, with particular emphasis on fatty acids related to the rumen metabolism. MFA, exclusively of dietary origin, i.e. C18:2, C18:3 and the C20 fatty acids, were excluded as input variables because their inclusion in the ANN might cause poor generalisation performance of the model for other dietary conditions. Oleic acid (*cis*-9 C18:1) was excluded due to its multiple origin in milk fat (Fievez et al., 2003). Oleic acid is endogenously de novo synthesised in the mammary gland or directly absorbed from the blood plasma after absorption from the small intestine or released from adipose tissue during negative energy balance periods. Hence, its concentration in milk fat depends also on lactation stage and activity of udder enzymes which can give generalisation problems of the model for early lactation cows. On the other hand, MFA of particular interest are the OBCFA (Table 3) because of their microbial origin in the rumen. The OBCFA pattern differs according to shifts in the rumen microbial population and environment (Vlaeminck et al., 2006b). These MFA were used by Vlaeminck et al. (2006a) for the development of a multi-linear regression model. To allow the performance of both the statistical and machine learning models to be compared, ANN models were developed using these FA as sole input variables. However, in the ANN models which will be discussed first, the C18-hydrogenation intermediates were also considered as possible input variables, because they are formed during saturation of poly-unsaturated fatty acids by rumen microbes and their accumulation depends on the presence of specific microbial communities and rumen conditions. Hence, these MFA include additional information about the rumen environment and its concomitant fermentation pattern. The first selection was further assessed during the modelling process by the method of the connection weights, resulting in a second and final selection of input variables (Table 3).

Table 3 – Milk fatty acids after final input variable selection (IVS) and milk odd and branched chain fatty acids (OBCFA) used by Vlaeminck et al. (2006a) in a multi-linear regression model for the prediction of rumen acetate, propionate and butyrate proportions

	Acetate		Propionate		Butyrate	
	IVS	OBCFA	IVS	OBCFA	IVS	OBCFA
Iso C14:0	x	x	x	x		
Iso C15:0		x		x	x	
Anteiso C15:0					x	x
C15:0	x	x	x	x	x	x
Iso C17:0	x			x		
Anteiso C15:0			x			
C17:0 + *cis*-9 C17:0	x	x	x	x	x	
Trans-10 C18:1	x		x		x	
Cis-9, *trans*-11 C18:2	x		x		x	
Trans-10, *cis*-12 C18:2					x	

3.2. *Model development*

Three training algorithms were used to train the ANN. The performance of the ANN for the prediction of acetate, calculated on the training and the independent test data, is given in Fig. 2. The Levenberg–Marquardt algorithm performs very well on the training data, but shows higher RMSE on the test data. Although a validation set was introduced to avoid overfitting of the training data, a significant difference occurred between the performance on the training and the test data. The reduced performance of the gradient descent algorithm compared to the other algorithms might be caused by the linear approximation of the error function, sometimes provoking poor convergence. The scaled conjugate gradient algorithm performs well, as for both test and training data there is no overfitting of the training data. Therefore, the scaled conjugate gradient algorithm has been chosen as learning algorithm for the ANN. There is no improvement in performance of ANN with more than 12 neurons in the hidden layer. The initial architecture of the ANN for the prediction of the acetate is therefore a feed-forward network with one hidden layer and 12 hidden nodes trained by the scaled conjugate gradient algorithm. For the prediction of propionate and butyrate, the same architecture has been chosen. A further selection of input variables was done based on the connection weights. For both acetate and propionate this resulted in a reduction from 13 to 6 MFA without a decrease in performance of the model. Seven input variables were retained in the model for butyrate prediction. During the reduction of the input variables, according to the connection weights method, the number of neurons has been gradually optimised each time one input variable was removed. The final models for the prediction of acetate, propionate and butyrate have six neurons in the hidden layer. The optimised weights and biases are given in Table 4.

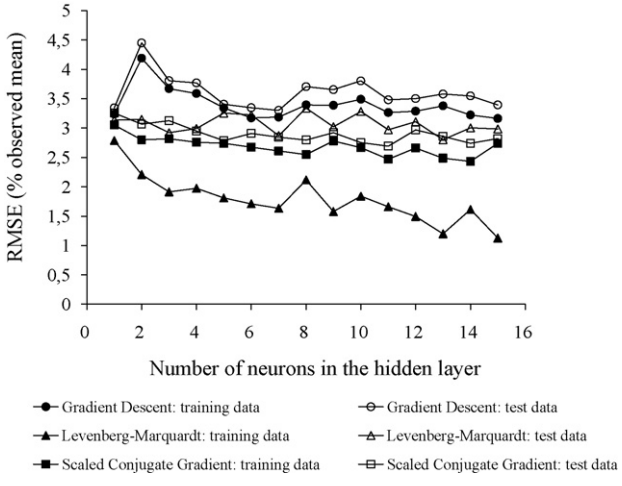

Fig. 2 – RMSE of ANN model predicting rumen acetate proportions developed using the gradient descent algorithm, the Levenberg–Marquardt algorithm and the scaled conjugate gradient algorithm for 1–15 neurons in the hidden layer.

The selected MFA (Table 3) are mainly OBCFA, formed by the microbial population in the rumen. These results are in accordance with the work of Vlaeminck et al. (2006a). The latter suggests that the variation in milk OBCFA is a reflection of shifts in the rumen microbial population due to dietary changes. Branched chain fatty acids (*iso* C14:0 and *iso* C15:0) are mainly formed by cellulolytic bacteria, like *Ruminococcus flavefacies* and *Ruminococcus albus*. An increase of these fatty acids can indicate an increased importance of cellulolytic bacteria resulting in a higher proportion of acetate, and a reduced proportion of propionate (Vlaeminck et al., 2006b). *Iso* C14:0 shows a positive connection weight and hence positively affects the prediction of acetate whereas negative connection weights and hence a negative correlation with propionate has been observed (Table 4). Amylolytic bacteria, like *Ruminobacter amylophilus* and *Succinivibrio dextrinosolvens*, which mainly produce propionate, show relatively lower levels of branched chain fatty acids (*iso* C14:0) and are enriched in linear odd chain fatty acids (C15:0) (Vlaeminck et al., 2004). *Iso* C17:0 has a negative influence on the prediction of acetate. In studies with varying dietary starch content or forage to concentrate ratios increased *iso* C17:0 proportions were associated with increased starch levels and decreased forage proportions (Vlaeminck et al., 2006b).

Further, *trans*-10 C18:1 and some conjugated fatty acids are retained in the models. Diets providing large amounts of readily digestible carbohydrates and reduced amounts of structural fibrous carbohydrates might provoke incomplete biohydrogenation resulting in an increase in milk *trans*-C18:1

Table 4 – Optimised weights and biases of the input and hidden layers of the artificial neural network and the connection weights of the input variables for the prediction of rumen acetate, propionate and butyrate proportions

Input layer	Hidden layer nodes						Connection weights
	1	2	3	4	5	6	
Acetate							
Iso C14:0	0.813	−0.587	0.771	−0.103	1.344	0.695	2.49
C15:0	0.225	−0.945	0.408	−0.691	−0.482	0.792	−0.167
Iso C17:0	1.15	0.835	−1.42	−1.16	−0.144	1.14	−0.71
C17:0 + *cis*-9 C17:1	0.527	1.10	−0.660	−0.420	0.422	−1.07	0.412
Trans-10 C18:1	−0.85	−0.612	0.268	0.238	−0.848	−0.498	−1.11
Cis-9, *trans*-11 C18:2	−1.69	0.783	0.064	1.514	−0.4	−0.215	−1.81
Hidden nodes bias	−1.84	1.05	−1.58	−0.106	1.44	1.57	
Output nodes weights	1.01	−0.38	0.478	0.324	0.992	−0.31	
Propionate							
Iso C14:0	1.12	−0.932	−0.547	0.378	−1.32	−0.387	−0.783
C15:0	−0.706	−0.617	−1.40	−0.855	0.097	−0.421	0.459
Anteiso C17:0	−0.457	0.535	1.32	−0.605	−0.536	−0.820	−0.756
C17:0 + *cis*-9 C17:1	−0.703	0.961	−0.390	0.581	−0.596	−0.715	−0.072
Trans-10 C18:1	0.906	0.282	0.964	−1.12	1.17	0.994	1.19
Cis-9, *trans*-11 C18:2	0.151	0.748	0.663	−0.962	0.288	−0.653	0.714
Hidden nodes bias	−2.13	1.57	0.177	0.248	−0.471	−2.18	
Output nodes weights	0.459	0.636	−0.573	−0.345	0.683	−0.032	
Butyrate							
Iso C15:0	0.577	0.699	0.100	−0.048	−1.91	0.352	0.141
Anteiso C15:0	0.483	−0.754	0.887	1.21	1.79	0.160	0.086
C15:0	−0.994	0.449	−0.527	−0.656	−0.333	−1.49	−0.552
C17:0 + *cis*-9 C17:1	0.680	0.954	−0.370	1.30	0.428	−0.157	0.537
Trans-10 C18:1	0.339	0.435	0.393	0.214	−0.283	1.20	1.04
Cis-9, *trans*-11 C18:2	−0.210	1.10	0.884	0.968	0.031	0.299	1.11
Trans-10, *cis*-12 C18:2	−0.830	−0.858	1.02	−0.173	−1.12	0.435	−1.25
Hidden nodes bias	0.438	1.17	0.488	−0.686	0.609	0.425	
Output nodes weights	−2.03	1.53	0.257	0.356	−0.440	1.67	

in milk fat (Bauman and Griinari, 2001). There is also a shift from the production of *trans*-11 C18:1 to a relatively higher production of *trans*-10 C18:1 by feeding diets high in grain/roughage ratio, which might explain the positive weight of *trans*-10 C18:1 on the prediction of propionate and its negative weight in the acetate model. The conjugated fatty acid *cis*-9, *trans*-11 C18:2 has a negative influence on the prediction of acetate and is positively related to propionate production. Both diet as well individual animal variations have a major influence on *cis*-9, *trans*-11 C18:2 in milk. Dietary factors that change the microbial processes in the rumen involve an alternation in the pathways of biohydrogenation that results in a change in the formation of *trans*-10 C18:1 and related intermediates like *cis*-9, *trans*-11 C18:2 and *trans*-10, *cis*-12 C18:2 (Bauman and Griinari, 2001). Diets high in fibrous carbohydrates result in more cellulolytic, acetate producing bacteria and a more extensive biohydrogenation towards C18:0 with less accumulation of C18-intermediates, including vaccenic acid, the precursor of *cis*-9, *trans*-11 C18:2 in the mammary gland.

3.3. *Model validation*

The results for the prediction of VFA proportions of the independent test data ($n = 35$) are given in Table 5 and Figs. 3 and 4. This table includes the results of the predictions, using all the MFA, the MFA after feature selection and the OBCFA, used in

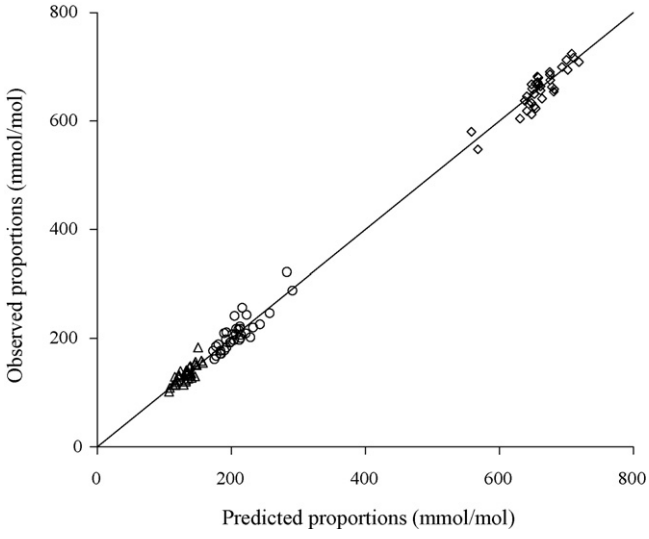

Fig. 3 – Prediction of the rumen proportions of acetate (◊), propionate (○) and butyrate (Δ) (mmol/mol volatile fatty acids) for the independent test data using ANN models based on both milk odd and branched chain MFA and biohydrogenation intermediates (g/100 g fat) (Table 3) ($n = 35$).

Table 5 – Performance of the ANN models to predict rumen proportions of acetate, propionate and butyrate (mmol/mol volatile fatty acids) for the test data ($n = 35$) using the mean square prediction error (MSPE)

	Acetate			Propionate			Butyrate		
	35 MFA[a]	6 MFA[b]	4 MFA[c]	35 MFA[a]	6 MFA[b]	5 MFA[c]	35 MFA[a]	6 MFA[b]	2 MFA[c]
Observed (mmol/mol)	658 ± 38.1	658 ± 38.1	658 ± 38.1	209 ± 33.5	209 ± 33.5	209 ± 33.5	133 ± 16.7	133 ± 16.7	133 ± 16.7
Predicted (mmol/mol)	657 ± 32.1	660 ± 32.9	658 ± 29.3	207 ± 25.2	208 ± 27.7	211 ± 28.9	131 ± 13.5	132 ± 13.4	131 ± 7.09
MSPE (mmol/mol)^2	262	305	489	231	257	372	129	101	150
RMSE (mmol/mol)	16.2	17.4	22.0	15.1	16.0	19.3	11.3	10.1	12.2
RMSE (% observed mean)	2.46	2.65	3.36	7.25	7.67	9.23	8.52	7.55	9.19
Proportion (%) of MSPE due to									
Mean bias	0.100	1.70	0.050	1.52	0.240	0.840	3.72	2.49	1.97
Line	2.09	0.300	0.500	9.10	0.960	0.650	1.01	0.007	19.1
Random	97.8	98.0	99.5	89.4	98.8	98.5	95.3	97.7	79.0

[a] ANN using all the 35 measured MFA (C4:0, C6:0, C8:0, C10:0, C12:0, *iso* C13:0, *anteiso* C13:0, *iso* C14:0, C14:0, *iso* C15:0, *anteiso* C15:0, *cis*-C14:1, C15:0, *iso* C16:0, C16:0, *trans*-C16:1, *iso* C17:0, *cis*-C14:1, *anteiso* C17:0, C17:0, *cis*-C17:1, C18:0, *trans*-6 C18:1, *trans*-9 C18:1, *trans*-10 C18:1, *trans*-11 C18:1, *cis*-9 C18:1, *cis*-11 C18:1, C18:2, *trans*-11 *cis*-15 C18:2, *cis*-9 *trans*-11 C18:2, *trans*-10 *cis*-12 C18:2, C18:3, C20:0, C20:1).
[b] ANN using the odd and branched chain MFA as well as biohydrogenation intermediates (Table 3).
[c] ANN using milk odd and branched fatty acids as included in the multi-linear regression model of Vlaeminck et al. (2006a) (Table 3).

the multi-linear regression model of Vlaeminck et al. (2006a) (Table 3).

In the model with both selected OBCFA and biohydrogenation intermediates the RMSE (% of the observed mean) is 2.65%, 7.67% and 7.61% for the prediction of acetate, propionate and butyrate, respectively, and is mainly due to random variation around the regression line (Table 6), whereas mean and line bias are only of minor importance. The total sum of the VFA is equal to 1000 mmol/mol. Lower absolute values of propionate and butyrate compared to acetate (observed mean 658, 209 and 133 for acetate, propionate and butyrate, respectively) result in a larger prediction error for these fatty acids as compared to acetate when expressed as percentage of the observed mean (RMSE).

When the results of the ANN with both OBCFA and biohydrogenation intermediates are compared to the predictions using the OBCFA as sole input variables, a small improvement in accuracy on the independent test data occurs (Table 5). The introduction of the C18-hydrogenation intermediates might therefore slightly improve the predictions of VFA. However, it is clear that the OBCFA are the most important MFA in the prediction process.

Comparing the results of these ANN models to these of an ANN including 35 MFA suggests that the most important and uncorrelated MFA were selected as there is no reduction in model performance after removal of input variables. In Fig. 4 the residuals are plotted against the predicted values. In none of the models, the line bias is significantly different from 0 which suggests that the developed models are unbiased.

3.4. *ANN versus a multi-linear regression model*

The choice for ANN or statistical techniques depends on the problem to be solved. For modelling data of low dimensionality or for approximating simple functions, classical techniques can be used (Basheer and Hajmeer, 2000). Vlaeminck et al. (2006a) derived multi-linear regression equations, developed with the same dataset used in the present study. Independent literature data, published in Shingfield et al. (2003, 2005) and Loor et al. (2005) were used to assess the generalisation capacity of this regression model, based on the milk OBCFA. Similarly, the performance of the ANN with OBCFA has been evaluated on this literature dataset, which allowed comparison of the different modelling techniques. The RMSE values

Table 6 – Validation of the ANN model developed in the present study and the multi-linear regression model (Vlaeminck et al., 2006a), both based on selected milk odd and branched chain fatty acids as input variables, using independent literature data, published in Shingfield et al. (2003, 2005) and Loor et al. (2005) ($n = 14$)

	Acetate		Propionate		Butyrate	
	ANN	MLR	ANN	MLR	ANN	MLR
Observed (mmol/mol)	684 ± 21.2	684 ± 21.2	196 ± 26.0	196 ± 26.0	120 ± 11.1	120 ± 11.1
Predicted (mmol/mol)	669 ± 10.8	670 ± 16.5	206 ± 13.3	199 ± 22.0	125 ± 8.5	124 ± 5.5
MSPE (mmol/mol)^2	426	420	543	312	101	114
RMSE (mmol/mol)	20.6	20.5	23.3	17.7	10.1	10.7
RMSE (% observed mean)	3.02	3.00	11.9	9.01	8.43	8.93
Proportion (%) of MSPE due to						
Mean bias	56.3	48.0	19.0	3.0	27.0	17.5
Line	8.14	0.700	0.130	2.70	2.73	0.600
Random	35.5	51.3	80.8	94.3	70.3	81.9

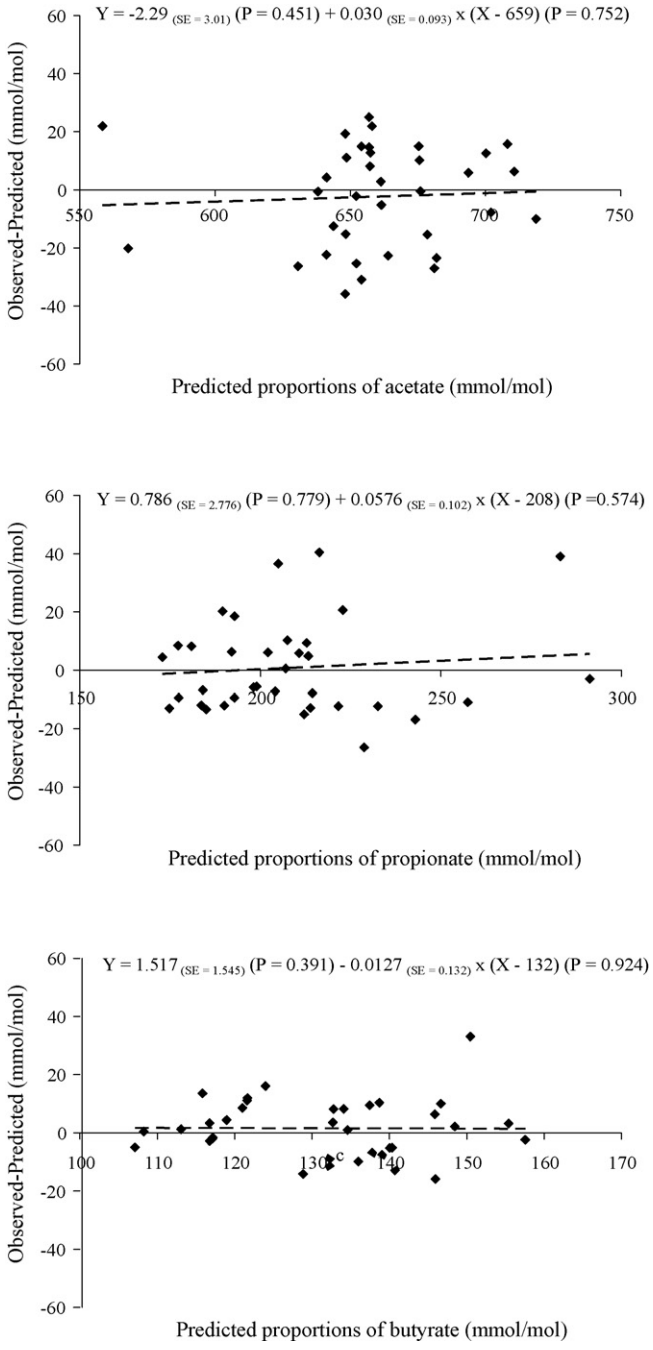

Fig. 4 – Plot of observed minus predicted molar proportions of acetate, propionate and butyrate vs. predicted molar proportions of acetate, propionate and butyrate (mmol/mol volatile fatty acids) for the independent test data using ANN models based on both milk odd and branched chain MFA and biohydrogenation intermediates (g/100 g fat) (n = 35).

for the regression model and the ANN are given in Table 6. When the results of the ANN are compared to those of the regression model, it is clear that the ANN does not perform better. The ANN underpredicts acetate (mean bias represents 56% of the error) and overpredicts propionate (mean bias represents 19% of the error), mainly for the data of the experiment of Shingfield et al. (2005). The biased results of the ANN on the data of Shingfield et al. (2005) might be due to the relatively high acetate and low propionate proportions, compared to the values in the original dataset used for model development (Table 1). In the experiment of Loor et al. (2005) linseed oil was supplemented to diets high and low in concentrate:forage ratio. Lipid supplementation to the diet is currently not included in the prediction model. The independent literature dataset could not be used to further evaluate the ANN model with OBCFA and hydrogenation intermediates as the latter fatty acids were not reported in detail in all three papers.

The ANN currently did not significantly improve the prediction compared to the statistical model, which suggests that no interactions or non-linear terms needed to be modelled (Özesmi et al., 2006). Therefore, it can be concluded that, under the circumstances included in the experiments used here, milk OBCFA are mainly linearly related to rumen VFA proportions. On the other hand, the ANN did not include any information about the origin of the data in the model and still seemed to perform as good as the multi-linear regression model of Vlaeminck et al. (2006a), in which experiment was included as a random factor in order to improve the estimation of the regression parameters. It seems that, compared to the multi-regression model, the ANN did not need experiment-related information to be included during the model development, in order to give similar results as the multi-regression model.

Further research will be conducted to improve prediction accuracy and use this prediction model in a wider range of diets and conditions.

4. Conclusions

The results of this study indicate that both the statistical and the machine learning methodology allow to develop predictive models for rumen VFA proportions, based on MFA patterns. The evaluation of the statistical regression model and the ANN model showed similar results. The ANN model currently did not significantly improve the prediction.

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