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On-farm evaluation of multiparametric models to predict subacute ruminal acidosis in dairy cows

M. Coppa [a], C. Villot [b,c], C. Martin [c], M. Silberberg [c,*]

[a] Independent Researcher, 10100 Turin, Italy
[b] Lallemand SAS, F-31702 Blagnac, France
[c] Université Clermont Auvergne, INRAE, VetAgro Sup, UMR 1213 Herbivores, F-63122 Saint-Genès-Champanelle, France

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ABSTRACT

This research aimed: (i) to evaluate on-farm (**FARM** data) multiparametric models developed under controlled experiment (**INRAE** data) and based on non-invasive indicators to detect subacute ruminal acidosis (**SARA**) in dairy cows. We also aimed to recover high discrimination capacity, if needed, by (ii) building new models with combined INRAE and FARM data; and (iii) enriching the models increasing from 2 to 5 indicators per model. For model enrichment, we focused on indicators determinable on-farm by quick and inexpensive routine analysis. Fifteen commercial dairy farms were selected to cover a wide range of SARA risk. In each farm, four Holstein early-lactating healthy primiparous cows were selected based on their last on-farm recording of milk yield and somatic cell count analysis. Cows were equipped with a reticulo-rumen pH sensor. The pH kinetics were analysed over a subsequent 7-day period. Relative pH indicators were used to classify cows with or without SARA. Milk, blood, faeces, and urine were collected for analysis of the indicators included in the models developed by Villot et al. (2020) on INRAE data that were externally evaluated using FARM data. Then, new models based on the same indicators were developed combining INRAE and FARM data to test whether a possible loss in performance was due to a limited validity domain of model by Villot et al (2020). Finally, the models developed combining INRAE and FARM data were adapted to the on-farm application and enriched by increasing indicators from 2 to 5 per model using linear discriminant analysis and leave-one-out cross-validation. The sensitivities (true-positive rate) in external evaluation on FARM data were substantially lower than those from cross-validation by Villot et al. (2020) (range: 0.1–0.75 vs 0.79–0.96, respectively), and the specificities (true-negative rate) showed a larger range with lower minimum values (range: 0.18–1.0 vs 0.62–0.97, respectively). The sensitivities of new models developed combining INRAE and FARM data ranged from 0.63 to 0.77. Models involving blood cholesterol, β-hydroxybutyrate, haptoglobin, milk and blood urea, and models involving milk fat/protein ratio, dietary starch proportion, and milk fatty acids had the highest performances, whereas models including sieved faecal residues and urine pH had the lowest. Enriching models to three indicators per model improved sensitivity and specificity, but the inclusion of more indicators was less or not effective. Larger field trials are required to validate our results and to increase variability and validity domain of models.

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Implications

Subacute ruminal acidosis is a diffuse disorder causing important economic losses in intensive dairy farming. Its on-farm detection is challenging, and we lack routinely applicable tools for this purpose. To detect subacute ruminal acidosis, we tested on-farm models, previously developed in controlled experiments, based on multiparametric non-invasive indicators. Models were built with diet, blood, milk, urine and faeces indicators monitored by inexpensive routine analysis. Our results confirm the potential of combinations of indicators in the on-farm detection of subacute ruminal acidosis in dairy cows, but model validation on a larger farms and cow number is recommended.

Introduction

Over the last few decades, dairy farming systems have been intensified to meet the rapidly growing worldwide demand for dairy products. In intensive dairy farms, cows are commonly fed

* Corresponding author.
E-mail address: mathieu.silberberg@inrae.fr (M. Silberberg).

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diets with disproportion between a high concentration of rapidly fermented carbohydrates (mainly starch) and low physically effective NDF to meet the energy requirements associated with their high milk yields. However, such diets may cause impaired ruminal health through variation in volatile fatty acid concentrations and decreased ruminal fluid pH, which lead to subacute ruminal acidosis (**SARA**) (Plaizier et al., 2008). There is no characteristic symptom in SARA–affected cows (Abdela, 2016), but several non-specific clinical signs may be present, like decreases in milk yield and milk fat content, lameness, reduction in feed intake, behavioural disturbances (i.e. drop of rumination, altered lying and ingestion time), etc. (Plaizier et al., 2008; Abdela, 2016; Zschiesche et al., 2022). Additionally, those non-specific clinical signs are often delayed and the cow's health is already altered when clinical signs become detectable. Some authors have also highlighted the correlation between SARA and several biomarkers of physiological status alteration or chronic inflammation: protein content and composition (i.e. haptoglobin), and mineral (like calcium, phosphorous, iron), glucose, urea, gas (like bicarbonate, CO_2 and O_2), hormones (like cortisol) and enzyme (like alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase) contents in blood (Plaizier et al., 2018; 2022; Antanaitis et al., 2019); pH and consistency in faeces (Abdela, 2016; Plaizier et al., 2018); pH, and NH_4+ in urine (Vagnoni and Oetzel, 1998). Changes in milk fat, protein and urea contents, fat/protein ratio (**FPR**), as well as in milk fatty acid (**FA**) composition have been linked to SARA (Comino et al., 2015; Jing et al., 2018; Zschiesche et al., 2022). Even though the association between SARA and the large range of indirect indicators previously illustrated has been consolidated, few studies have tried to use them to predict the occurrence of SARA in dairy cows. Recently, Khorrami et al. (2021) developed models using single indicators of milk and diet composition, but only milk urea content among the tested milk constituents seemed to be correlated with ruminal pH. Mensching et al. (2020) developed multiparametric models describing the relationship between reticulo-ruminal pH parameters (average pH, time at pH < 5.8 and ΔpH) and several indicators of milk composition (i.e. fat, protein, lactose contents) and of diet composition (ether extract, starch, physically effective NDF). Only Villot et al. (2020) have developed predictive models from behaviour (DM intake, drinking acts, rumination time), blood (cholesterol, bicarbonate, β-hydroxybutyrate (**BHBA**), glucose), urine pH, milk (FPR, urea, FA composition), and faeces (pH and sieving residuals) aiming at discriminating cows affected or not by SARA. Villot et al. (2020) developed 18 different models combining 2–3 indicators from different matrices. Models by Villot et al. (2020) were logit function predicting the probability (y) of a cow to be affected or not by SARA (y > 0.5 = SARA occurrence); they were able to accurately detect SARA with high sensitivity and specificity (respectively ranging from 79 to 100% and from 61.5 to 100%, depending on the model). The best-performing models (precision > 90%) included variables from blood (bicarbonate and BHBA), milk (milk urea, FPR), and particularly FA composition (n-6 FA and C18:1trans10/C18:1trans11 ratio) and behaviour (DM intake (**DMI**) and the number of drinking acts) (Villot et al., 2020). However, the models by Villot et al. (2020) were developed using data from SARA induced experimentally with a unique and specific diet. A wide range of diets with a high risk of SARA are used on-farm and individuals fed the same diet can differ in SARA susceptibility, due to metabolic specificity or feeding behaviour and social hierarchy (Khiaosa-ard et al., 2018). We hypothesised that the models developed by Villot et al. (2020) have a lower discrimination capacity when applied on-farm probably because the calibration dataset (controlled experiment) and the evaluation dataset (on-farm) are heterogeneous, as the on-farm data would probably be out of the validity domain of the models by Villot et al., (2020). Therefore, one aim of our research was (i) to evaluate on-farm the models developed by Villot et al. (2020) based on combinations of indicators used to detect SARA in dairy cows under controlled trial conditions. We also aimed at recovering high discrimination capacity, (ii) by building new models with data merged from controlled experiment and on-farm trial, to extend their validity domain and (iii) by enriching such new models by increasing the number of indicators from 2 to 5. For model enrichment, we focused on indicators determinable on-farm and by quick and inexpensive routine analysis.

Material and methods

Animals, diets and experimental procedure

The present study was carried out using data from one controlled experiment (**INRAE**) and one trial on commercial farms (**FARM**) carried out between 2016 and 2017 in which, dairy cows were housed in free stall barns and had free access to water. The first experiment (INRAE; described in detail by Villot et al., 2018; 2020) was conducted at the dairy cow research facilities of the farm of Herbipôle (INRAE, Theix, France, https://doi.org/10.15454/1.5572318050509348E12). Eleven primiparous Holstein dairy cows (mean ± SE: 135 ± 7 days in milk; 27.5 ± 2.3 kg/day milk yield at the beginning of the experiment) were fed successively a low starch diet (13% of diet DM) or a high starch diet (35% of diet DM) as described by Villot et al. (2018 and 2020).

The second trial (FARM) was carried out on commercial farms for two weeks.

This trial was conducted on 15 commercial dairy farms of the Holstein cows located in the north-west of France, aiming to cover a large variability of farming practices similar to those observed in literature for the same territory (Hurtaud et al., 2014). Their risk of SARA was evaluated according to the parameters proposed by Sauvant and Peyraud (2010): FPR (<1.2), urea of tank milk (<250 mmol/mL) from the last milk control recording; percentage of concentrate in the diet (>50% DM), NDF (<35% DM) and rumen digestible starch proportion (>25% DM) in dairy cow diet. As we aimed to cover a wide range of risks of observing cows with SARA, in relation to farming practices, we privileged farms with parameters below or above these thresholds. The characteristics of the selected farms are given in Table 1. Early-lactating primiparous cows were considered to be the most susceptible to SARA (Humer et al., 2015); thus, in each farm, four of them were randomly selected (Microsoft Excel "random" function) from among those in the first part of their lactation (days in milk [**DIM**] between 20 and 159, only three of them were below 30 DIM). Based on the monthly veterinarian inspection, the selected cows were healthy and had not presented any apparent clinical health status alteration, in particular lameness during the last 6 months or mastitis (somatic cell count was used as an indicator) during ongoing lactation. The characteristics of the selected cows are given in Table 1.

Sampling and analysis

Diet

Diet composition was established by the experimental protocol in INRAE experiment and was characterised by on-farm survey in FARM trial. In INRAE experiment, the total mixed ration was offered twice a day (in the morning and the afternoon), whereas in the FARM trial, the total mixed ration was offered between 1 and 3 times a day, depending on the farm. In each study, two representative sub-samples of the offered total mixed ration were collected once a week for INRAE experiment and only once on the day

Table 1
Characteristics of the farms investigated and of the monitored primiparous dairy cows (FARM data).

Item	Average	Median	Min	Max	SD
Characteristics of the 15 farms					
Dairy cow, n	73	62	33	135	27.4
Age at first calving, month	26	27	24	30	1.6
Milk yield, ×1 000 L/year	695	670	174	1 200	272.2
Return rate, %[1]	33.6	35.0	17.0	50.0	8.70
Culling rate, %	34.2	35.0	20.0	50.0	8.77
Meals, n/day	1.3	1.0	1.0	3.0	0.59
Lameness, n/year	19.4	12.0	0.0	77.0	22.44
Mastitis, n/year	20.0	20.0	3.5	50.0	12.18
Particle size of diet, %					
Sieve 5 mm	41	44	22	56	10.6
Sieve 2 mm	39	38	24	64	11.2
Sieve 1 mm	18	18	13	26	4.1
Pan	2	2	0	6	1.5
Ingredients (% DMI)					
Maize silage	53.0	55.6	28.5	69.2	11.38
Grass silage	12.3	12.0	0.0	40.8	10.08
Hay	2.5	0.0	0.0	20.5	5.26
Straw	2.0	0.0	0.0	13.3	3.78
Pasture	0.9	0.0	0.0	12.9	3.34
Total forage	72.6	73.9	52.1	80.7	7.42
Minerals	1.5	1.8	0.0	2.5	0.69
High moisture corn silage	4.0	4.3	0.0	11.3	4.32
Cereal grains	2.5	0.0	0.0	11.5	4.06
Extruded legume oilseeds	7.7	6.5	0.0	21.3	8.61
Commercial concentrate mix	22.4	24.3	7.6	30.8	7.14
Total concentrates	27.4	26.1	19.3	47.9	7.42
Diet DM (%)	53.0	55.6	28.5	69.2	11.4
Diet nutrient composition (%, DM)[2]					
CP	14.4	14.2	11.7	17.8	1.8
NDF	37.6	37.3	32.8	40.4	2.0
Physically effective NDF > 8 mm	15.3	16.0	8.6	22.5	4.0
Physically effective NDF > 2 mm	30.1	30.6	26.4	33.5	2.3
Physically effective NDF > 1 mm	36.9	37.0	32.7	39.6	2.0
ADF	20.5	20.2	17.7	22.4	27.00
Starch	23.3	22.7	20.2	28.1	23.04
Other carbohydrates	6.6	6.1	5.9	7.4	9.80
Characteristics of the 59 monitored primiparous cows					
DIM	85	85	20	159	40.1
Milk yield, Kg/cow*day	30.8	30.8	18.6	43.2	4.78
Milk fat content, g/Kg	38	38	23	57	6.7
Milk protein content, g/Kg	30	31	24	37	2.5
FPR	1.3	1.2	0.8	2.2	0.25
Somatic cell count, n × 1 000/mL	69	44	11	442	77.7
Milk urea, mmol/L	4.7	4.8	1.2	7.2	1.4

Abbreviations: DIM = days in milk; DMI = DM intake; FPR = fat/protein ratio.
[1] Return rate = ratio of number of heifers / number of productive dairy cows.
[2] Determined by laboratory chemical analysis.

of sampling for the on-farm trial. One sample was analysed to determine DM (60 °C for 48 h), NDF, ADF (Van Soest et al., 1991), CP (method 968.06; AOAC, 2005) and starch (Faisant et al., 1995) contents. The other sample was wet sieved to evaluate particle size proportions as detailed by Villot et al. (2020). The physically effective NDF was estimated by multiplying the NDF concentration of the total mixed ration by the proportion of particles: >8 mm, >2 mm, and >1 mm (Kononoff et al., 2003), as follows: [NDF (%DM)/100] × [particle size of the diet > n mm (%)/100]; in which n = 8, 2 or 1 mm.

Kinetics of reticulo-rumen parameters and drinking acts

Reticulo-rumen (**RR**) pH and temperature were monitored continuously throughout both INRAE and FARM studies using a wireless sensor (eCow, Exeter, UK), as described by Villot et al. (2018). The sensors were calibrated by heating in a water bath at 39 °C and calibrating against pH 4 and 7 standard buffers before insertion. Calibration was checked overnight using a pH 6.86 buffer. Each RR sensor was set up to record mean pH over 15 min. The RR sensor was orally administered to the animals on sampling day, just after sampling of blood, faeces and urine, before the total mixed ration morning feeding. The pH kinetics were analysed over the following seven consecutive days, and daily relative RR pH indicators were calculated. Signal processing was applied to raw pH values in order to calculate relative pH indicators (**NpH**) by filtering and normalising data to remove inter-individual variability, sensor drift and sensor noise (Villot et al., 2018). Both the sensitivity and specificity of NpH indicators by Villot et al. (2018) ranged between 0.82 and 0.88. Accordingly, a cow was considered as SARA positive (SARA+) if NpH decreased by more than 0.3 for more than 50 min, and NpH range varied by more than 0.8 and/or its SD was above 0.2.

The number of daily drinking acts was estimated based on a drop in RR temperature of at least 0.2 °C compared to the individual daily mean RR temperature (Gasteiner et al., 2015) within a 30-min interval (Villot et al., 2020).

Milk

Milk yield was recorded automatically at each milking in each farm either via a milking robot (four farms) or in a milking parlour

(11 farms + INRAE experimental farm). Milk samples were collected over two consecutive milkings (evening and morning) the day after the oral administration of the RR sensor. A fresh subsample was stored at +4 °C with potassium dichromate (Merck Chimie SAS, Fontenay-sous-Bois, France) and analysed for fat, protein and urea content and somatic cell count by mid-infrared spectroscopy (MilkoScan 4000; Foss Electric A/S, Hillerod, Denmark; IDF, 2008; 2013) within 24 h of sampling. Daily milk composition was calculated based on the individual milk yield of morning and evening milking. A second subsample without preservative was freeze-dried (Thermovac TM-20, Froilabo S.A., Meyzieu, France) for FA analysis by gas chromatography according to Ferlay et al. (2010).

Blood

Blood samples were individually collected once on the day of the oral administration of the RR sensor from the jugular vein into a lithium heparinised 10-mL tube (Elvetec Services, Meyzieu, France) before the total mixed ration morning feeding (Hussein et al., 2020), to make them homogeneous with those collected during INRAE experiment (Villot et al., 2020). Blood pH and blood gases were immediately measured with a blood gas analyser (ABL5, Radiometer, Copenhagen, Denmark). Another 10-mL blood sample was collected at the same time using EDTA-collecting tubes (Elvetec Services, Meyzieu, France), and plasma was separated immediately after sampling by centrifugation (3 500*g*, 15 min, +4 °C). Plasma samples were frozen at −20 °C until analysis. Urea, BHBA, NEFA (Kit NEFA-HR2, Fujifilm WAKO), glucose, aspartate aminotransferase, gamma-glutamyl transferase and alkaline phosphatase were determined in plasma on an Arena 20XT (Thermo Scientific, Vaanta, Finland) automated analyser as described by Villot et al (2020). Cholesterol, interleukin-1β, interleukin-6, minerals, protein composition (total protein, albumin, globulin, bilirubin and haptoglobin) and oxidative status (advanced oxidation protein product, reactive oxygen metabolites, ferric-reducing antioxidant power, paraoxonase) were analysed at 37 °C by means of a clinical autoanalyser (ILAB 650, Wefen, Instrumentation Laboratory, Lexington, MA, USA). The red and white blood cell count, platelet count, haemoglobin, haematocrit, mean corpuscular volume, lymphocytes, monocytes, granulocytes and eosinophils were analysed at 37 °C by means of a clinical autoanalyser (ScilVet ABC, Scilvet, France) specific for cattle.

Faeces and urine

Collection of urine and faecal samples (500 mL each) was synchronised with blood collection. Faecal samples were collected from the rectum of each animal, and faecal pH was immediately measured with a digital pH-meter (VWR pH100) with a precision of 0.1 unit, calibrated with standard solutions (pH = 4, 7 and 10). Faecal density was calculated by weighing a 425 mL faecal sample, which was then wet sieved to evaluate faecal particle size proportions as detailed by Villot et al. (2020). Urine was collected during spontaneous urination before the morning feed distribution, and pH was immediately recorded using the same instrument and procedure as described for faeces.

Statistical analysis

A total of 79 variables were obtained from seven different matrices (diet, behaviour, rumen, faeces, blood, milk, and urine) and then statistically treated using SAS (Version 9.4; SAS Institute, 2009). When necessary, data were log-transformed before analysis (details given in Supplementary Table 1). The main steps of our work with the related statistical procedures are summarised in Fig. 1. The SAS code of the statistical procedure applied is reported in the Supplementary Material 1.

Step 1: Evaluation of models developed by Villot et al. (2020) using FARM data

The models by Villot et al. (2020) are detailed in the Supplementary Table 1. An external evaluation of the multiple logit functions models published by Villot et al. (2020) was performed with Excel (Microsoft Office 365®, 2013) by applying them to the FARM data only to predict the probability (y) of a cow to be affected by SARA (y > 0.5). Models 1, 3, 6, 8 and 17 by Villot et al. (2020 including DMI as an indicator were not tested as it was not possible to measure DMI on-farm.

Step 2: Development of new models using combined INRAE and FARM data, and based on indicators from Villot et al. (2020)

In an attempt to understand whether a possible lower discrimination capacity was due to (i) non-robust indicators included in the models, or (ii) heterogeneity in datasets because FARM data would be out of the validity domain of the models by Villot et al. (2020), a principle component analysis ("proc princomp" of SAS) was first performed on the combined controlled INRAE and FARM data. The principle component analysis was based on the indicators used for the external evaluation of the models by Villot et al. (2020). Hotelling's T 2 test was performed during the principle component analysis, selecting a 95% confidence region. Secondly, the same indicators included in each model by Villot et al (2020) were used to build new models. A linear discriminant analysis ("proc discrim" of SAS) with leave-one-out cross-validation on the combined INRAE and FARM data was performed.

Step 3: On-farm adaptation and enrichment of the models

To test if it was possible to further improve discrimination capacity, the LDA models from step 2 were also enriched by increasing the number of variables from 2 or 3 to 4 and 5. The enriched models obtained by LDA were adapted to the on-farm application by using only indicators quickly determinable on-farm during a visit or through inexpensive and rapid routine analysis, using two types of variables (referred to the individual cow or giving information related to the herd diet, as a further risk factor). Additional variables to be added to the initial models of Villot et al (2020) were selected following three steps:

1. an ANOVA ("proc glm " of SAS) was applied to each indicator, using the SARA group (SARA+ or SARA−) as a fixed factor to determine whether it was affected or not by SARA. A first and broad selection of variables was performed by retaining only those showing differences in *P*-value < 0.10.
2. a second selection of the most discriminant variables was made by combining two approaches: (i) correlation matrix ("proc corr" of SAS) to establish the correlations between variables, (ii) a partial least squares discriminant analysis ("proc pls method = pls cv = one" of SAS) to rank the importance of the variables in the discrimination of SARA+ and SARA− cows, following the same procedure used by Villot et al. (2020). Variable importance in projection scores of the partial least squares discriminant analysis estimates the importance of each variable in the projection to the latent structure. Only those with the highest variable importance in projection among variables from the same matrix correlated with Pearson's $r > |0.7|$ were kept for the further steps of model enrichment. Furthermore, only variables with variable importance in projection close to or higher than 1 were considered. The last criterion for variable selection was feasibility on farm, so an indicator was kept if measurable using inexpensive routine laboratory analysis. Therefore, the milk FA profile was not used for model enrichment since its acquisition by gas chromatography is time-consuming. Only saturated FA (**SFA**) milk concentration was kept, as its estimation by mid-

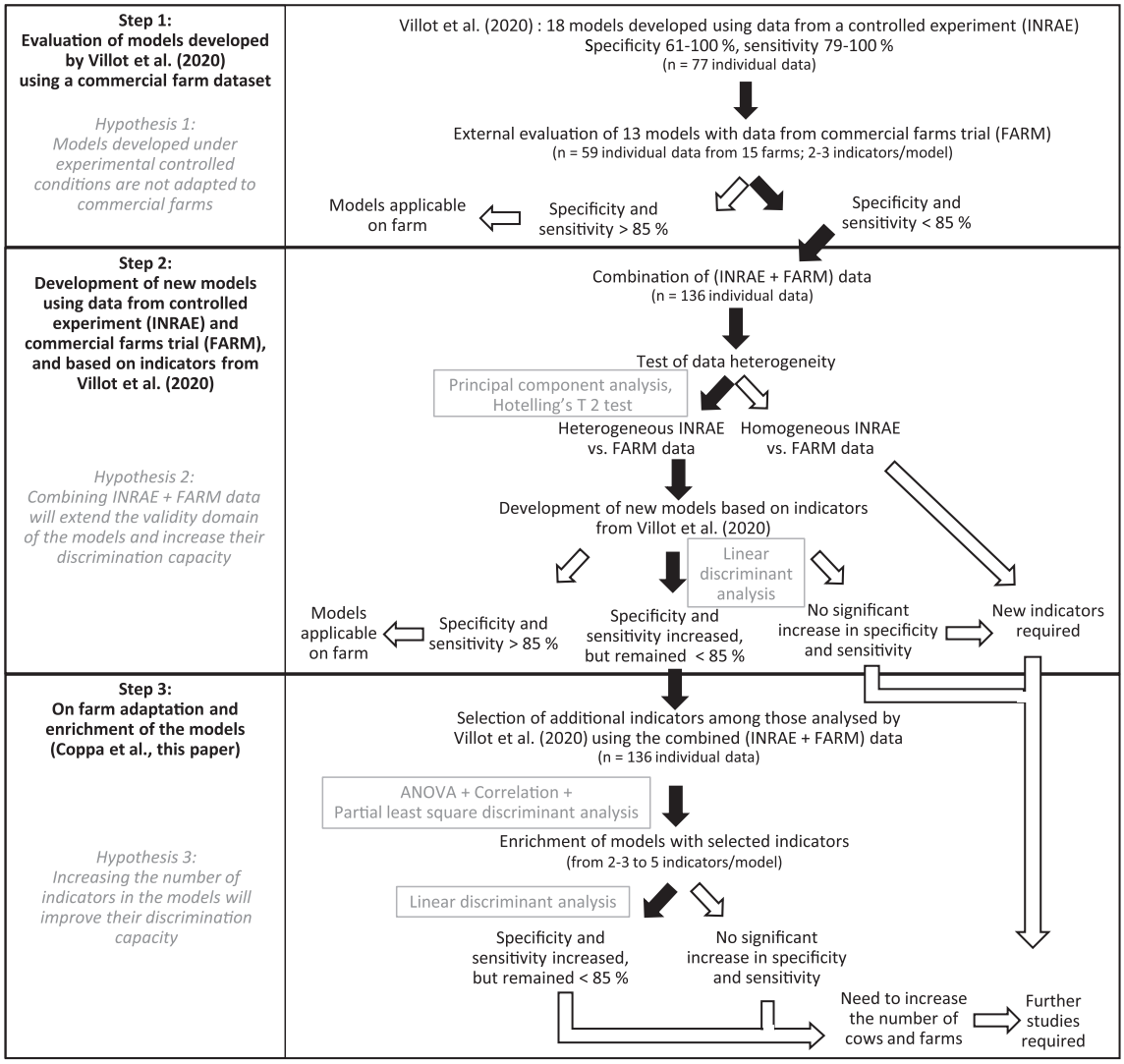

Fig. 1. Summary of the main steps of this work and the related statistical procedures used for the on-farm evaluation of multiparametric models to predict subacute ruminal acidosis in dairy cows.

Table 2
External evaluation using on-farm data (FARM) of the models developed under controlled trial conditions by Villot et al. (2020) to detect subacute ruminal acidosis using non-invasive indicators in dairy cows.

Model Villot et al. (2020)	Model variables						On-farm external evaluation (%)[1]			
No.	Matrix	Var 1	Matrix	Var 2	Matrix	Var 3	Se	Sp	Pr	Ac
2	Blood	Cholesterol, mmol/L	Milk	n-6 FA g/100 g FA			27 ± 1.8	92 ± 1	66 ± 4.2	67 ± 1.2
4	Blood	HCO_3, mmol/L	Milk	Urea, mmol/L			57 ± 2.7	73 ± 2	61 ± 2.9	66 ± 1.6
5	Blood	HCO_3, mmol/L	Milk	FPR			65 ± 2.4	59 ± 2.1	51 ± 2.7	61 ± 1.4
7	Blood	BHBA, mmol/L	Milk	Urea, mmol/L			15 ± 1.6	100 ± 0.0	95 ± 4.9	62 ± 2
9	Blood	BHBA, mmol/L	Blood	Glucose, mmol/L	Urine	pH	14 ± 1.7	100 ± 0.0	95 ± 4.9	62 ± 1.9
10	Milk	Urea, mmol/L	Urine	pH			48 ± 2.7	70 ± 1.8	56 ± 2.6	60 ± 1.7
11	Behaviour	Drinking act, No/day	Faeces	pH	Milk	Urea, mmol/L	63 ± 2.0	78 ± 1.4	68 ± 2.4	71 ± 1.0
12	Behaviour	Drinking act, No/day	Faeces	pH	Milk	SFA, g/100 g FA	100 ± 0.0	0 ± 0.0	39 ± 1.9	39 ± 1.9
13	Faeces	pH	Blood	BHBA, mmol/L			10 ± 1.4	100 ± 0.0	95 ± 4.9	64 ± 1.9
14	Faeces	pH	Milk	Urea, mmol/L			31 ± 2.2	84 ± 1.2	59 ± 2.5	61 ± 1.6
15	Faeces	pH	Milk	C18:1 tr10/tr11, log g/100 g FA			39 ± 1.8	80 ± 1.9	57 ± 2.7	64 ± 1.6
16	Faeces	pH	Milk	FPR			53 ± 2.5	79 ± 1.4	63 ± 2.3	69 ± 1.1
18	Faeces	Sieving residue, 5 + 2 mm, %	Urine	pH			76 ± 2.2	18 ± 1.8	38 ± 1.9	41 ± 1.6

Abbreviations: BHBA = β-hydroxybutyrate; C18:1 tr10/tr11 = C18:1 trans10/C18:1trans11 ratio; FA = fatty acids; FPR = fat/protein ratio; SFA = saturated FA Var = variable.
[1] Se = sensitivity; Sp = specificity; Pr = precision; Ac = accuracy; Mean from bootstrap procedure ± SE.

infrared spectroscopy gives results similar to those of gas chromatography (Coppa et al., 2017).
3. Enriched LDA ("proc discrim" of SAS) models were built by adding 1, 2 or 3 indicators to those proposed in the models of Villot et al. (2020). As results, LDA models with 3, 4 or 5 variables were tested.

Models performance evaluation

For the evaluation of the discrimination capacity of all models, the sensitivity (calculated as the true-positive rate), specificity (calculated as the true-negative rate), precision (calculated as the true positives over the true + false positives), and accuracy (calculated as the ratio between the true positives + the true negatives and the total samples) were used, according to Fawcett (2006). Accordingly, in the current study, the sensitivity and specificity express the error rate within a group to be discriminated (SARA+ or SARA−, respectively), whereas the precision expresses the capacity of the model to detect the samples, respecting a criterion over all samples, and the accuracy expresses the reliability of the model (Fawcett, 2006). The SE of sensitivity, specificity, precision and accuracy of both the external evaluation of the model by Villot et al. (2020) and the calibration and cross-validation of each new linear discriminant model were estimated using a bootstrap resampling procedure ("proc surveyselect" of SAS). In particular, a random resampling with substitution ("outhits method = urs" of SAS) of a sample of the same extent as the original dataset

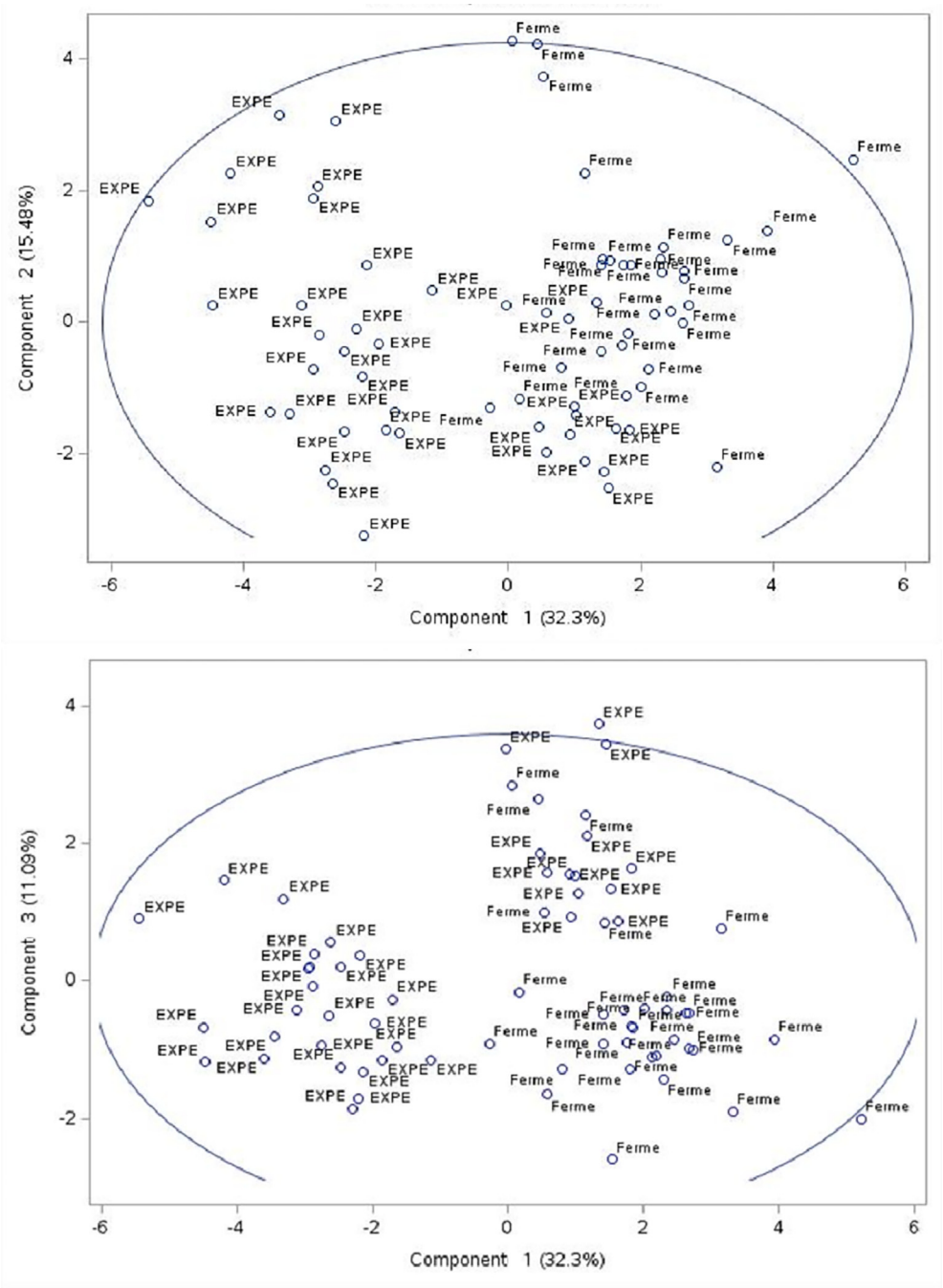

Fig. 2. Representation of the distribution of the individuals from three principal components analysis performed on the indicators of diet, behaviour, milk, blood, urine and faeces used for the development of models to predict subacute ruminal acidosis in dairy cows. EXPE = data from the controlled experiment (INRAE) conducted at the experimental farm; Ferme = data from commercial farms (FARM). The ellipse represents the 95% confidence region determined by Hotelling's T 2 test.

Table 3
Performance of subacute ruminal acidosis detection in dairy cows of linear discrimination models developed combining controlled experiment (INRAE) and on-farm trial (FARM) data based on the indicators included in the models of Villot et al. (2020).

Model Villot et al. (2020)	Linear discriminant model variables						Model coefficients				Leave-one-out cross-validation performance of the models (%)[1]			
No.	Matrix	Var 1	Matrix	Var 2	Matrix	Var 3	K	X1	X2	X3	Se	Sp	Pr	Ac
2	Blood	Cholesterol, mmol/L	Milk	n-6 FA, g/100 g FA			−4.28	−0.33	2.59		71 ± 2.0	88 ± 1.8	85 ± 2.3	80 ± 1.9
4	Blood	HCO_3, mmol/L	Milk	Urea, mmol/L			−9.12	0.23	0.59		76 ± 1.5	78 ± 1.6	78 ± 1.8	77 ± 1.4
5	Blood	HCO_3, mmol/L	Milk	FPR			−10.6	0.26	2.61		68 ± 1.5	81 ± 1.9	78 ± 2.1	75 ± 1.5
7	Blood	BHBA, mmol/L	Milk	Urea, mmol/L			−4.13	1.36	0.82		79 ± 1.1	69 ± 2.2	73 ± 1.8	74 ± 1.6
9	Blood	BHBA, mmol/L	Blood	Glucose, mmol/L	Urine	pH	−16.5	2.85	−1.62	2.02	69 ± 1.6	59 ± 1.9	64 ± 1.6	64 ± 1.5
10	Milk	Urea, mmol/L	Urine	pH			−18.0	1.76	0.93		75 ± 2.3	79 ± 1.9	80 ± 2.3	77 ± 2.0
11	Behaviour	Drinking act, n°/d	Faeces	pH	Milk	Urea, mmol/L	−15.0	0.18	1.66	0.76	82 ± 1.2	83 ± 1.0	85 ± 1.1	82 ± 1.0
12	Behaviour	Drinking act, n°/d	Faeces	pH	Milk	SFA, g/100 g FA	−23.4	0.002	2.57	0.10	69 ± 1.4	74 ± 1.9	74 ± 2.1	72 ± 1.6
13	Faeces	pH	Blood	BHBA, mmol/L			−15.7	1.92	2.28		69 ± 0.9	74 ± 1.3	72 ± 1.3	71 ± 0.9
14	Faeces	pH	Milk	Urea, mmol/L			−13.7	1.67	0.70		77 ± 1.4	80 ± 1.0	79 ± 1.0	79 ± 0.9
15	Faeces	pH	Milk	C18:1 tr10/tr11, log g/100 g FA			11.9	−1.94	0.57		69 ± 0.9	85 ± 1.1	81 ± 1.3	78 ± 1.0
16	Faeces	pH	Milk	FPR			−17.0	2.06	3.28		72 ± 0.9	75 ± 1.6	73 ± 1.7	73 ± 1.0
18	Faeces	Sieving residue, 5 + 2 mm, %	Urine	pH			−29.1	0.07	3.45		63 ± 1.8	58 ± 2.0	61 ± 1.4	61 ± 1.6

Abbreviations: BHBA = β-hydroxybutyrate; C18:1 tr10/tr11 = C18:1 trans10/C18:1trans11 ratio; FA = fatty acids; FPR = fat/protein ratio; K = constant; SFA = saturated FA; Var = variable; Xn = coefficient of variable n.
[1] Se = sensitivity; Sp = specificity; Pr = precision; Ac = accuracy; Mean from bootstrap procedure ± SE.

was generated 20-fold. For each subsampled dataset, the external evaluation or calibration and cross-validation procedure was iterated and the average sensitivity, specificity, precision and accuracy and their SE were calculated (Efron and Tibshirani, 1993). For each step, the target for each model was to reach values > 85% for both sensitivity and specificity, considered a reasonable threshold to allow a possible careful use for individual screening on farm (De Marchi et al., 2014; Villot et al., 2020; Coppa et al., 2021).

Results

Reticulo-rumen parameters and rumen, faeces, blood, milk, urine, behaviour, and diet indicators

Descriptive statistics for rumen, faeces, blood, milk, urine, behaviour, and diet indicators from combined INRAE and FARM data are presented in Supplementary Table 2. The NpH times spent below < −0.3 and below < −0.5 ranged from 0 to 539 min and from 0 to 285 min, respectively. The NpH range varied from 0.3 to 1.12 and the NpH SD from 0.09 to 0.39. Forage/concentrate ratio ranged from 80/20 to 46/54 and starch proportion from 13 to 35% of diet DM. Variability in rumen NpH and in cow diet was reflected by a large variability in most of the blood, milk, faeces and behaviour indicators. For instance, milk FPR ranged from 0.49 to 2.15, urea from 1.20 to 7.16 mmol/L and SFA from 47.3 to 75.3 g/100 g FA. In blood, bicarbonate and BHBA ranged from 16.0 to 39.0 and from 0.15 to 1.49 mmol/L, respectively. In faeces and urine, pH ranged from 5.6 to 7.3 and from 7.1 to 8.7, respectively.

Evaluation of models developed by Villot et al. (2020) using FARM data

Among the 59 cows monitored on farms, 22 were classified as SARA+ and 37 as SARA−, based on the NpH indicators (Villot et al., 2018).The prevalence of SARA based on NpH indicators

Table 4
Variable importance in projection scores of each indicator calculated with partial least squares discriminant analysis used to rank the importance of the variables in the detection of subacute ruminal acidosis in dairy cows.

Matrix	Indicator	Variable importance in projection
Diet	Forage, % DM	2.0
Diet	Starch, % DM	2.0
Milk	n-6 FA, g/100 g FA	1.6
Blood	HCO_3, mmol/L	1.6
Blood	pH	1.6
Milk	C18:1 trans10/ trans11, log g/100 g FA	1.5
Milk	SFA, g/100 g FA	1.4
Blood	BHBA, mmol/L	1.2
Milk	FPR	1.2
Urine	pH	1.1
Blood	Hp, log g/L	1.0
Milk	Urea, mmol/L	1.0
Blood	Urea, mmol/L	1.0
Faeces	Density, kg/m^3	1.0
Blood	Platelets, log n/mL	0.7
Faeces	pH	0.6
Blood	Cholesterol, mmol/L	0.6
Milk	Somatic cell count, log n × 1 000/mL	0.6
Blood	Globulin, g/L	0.5
Blood	Cl, mmol/mL	0.5
Blood	Aspartate aminotransferase, n/L	0.5
Blood	Glucose, mmol/L	0.4
Blood	Albumin, g/L	0.4
Milk	Iso-FA, g/100 g FA	0.3
Blood	Alkaline phosphatase, log n/L	0.3
Blood	Monocytes, 10^3/mm^3	0.2
Blood	Partial pressure of CO_2, mm Hg	0.2

Abbreviations: BHBA = β-hydroxybutyrate; FA = fatty acids; Hp = haptoglobin; FPR = fat/protein ratio; SFA = saturated FA.

across herds was as follows: 4/4 cows: 1 herd; 3/4 cows: 2 herds; 2/4 cows 3 herds; 1/4 cow: 6 herds; 0/4 cows: 3 herds. When evaluating the on-farm models developed by Villot et al. (2020) (Table 2), the sensitivity was below 50% for most of them. When sensitivities above 75% were reached (model 18: sieved faecal residue 5 + 2 mm – urine pH), the specificity was very low (below 20%) and vice versa. Only model 4 (blood bicarbonate – milk urea), model 5 (blood bicarbonate – milk FPR), model 11 (drinking acts – faeces pH – milk urea), and model 16 (faeces pH – milk FPR) showed both sensitivity and specificity above 50%, but below 75%. The accuracy was below 70% for all the models. The individuals of controlled trials appeared clearly separated from individuals of commercial farms (Fig. 2) in the principle component analysis performed on the indicators of diet, behaviour, milk, blood, urine and faeces used for the development of models by Villot et al. (2020), and independently of the SARA group.

Development of new models using combined INRAE and FARM data, and based on indicators from Villot et al. (2020)

When linear discriminant models were built using the combined INRAE and FARM data with the same variables as the models of Villot et al. (2020), sensitivity was always above 62% and above 70% for models 2, 4, 7, 10, 11, 14, and 16 (Table 3). The specificity was above 60% for all the models (except for models 9 and 18) and was above 75% for models 4, 5, 10, 14, 15 and 16. Models 2, 5, 11 and 15 reached a specificity above 80%, but the sensitivity was below 75% for models 5 and 15. Models 4, 10, 11 and 14 showed the best compromise between sensitivity and specificity, both of which, along with precision and accuracy, were above 75%.

On-farm adaptation and enrichment of the models

A total of 41 among 79 indicators showed statistically significant differences between SARA+ and SARA− cows (Supplementary Table 3), and a large majority of them were measured in blood (41% of total modified indicators) and milk (34%). Only 14 indicators had a variable importance in projection from a partial least squares discriminant analysis close to or higher than 1: five indicators related to milk, five to blood, two to diet, and one to urine and faeces (Table 4). However, only 12 indicators were kept for model enrichment as they were easily measurable on-farm. Diet indicators showed the highest variable importance in projection, followed by blood bicarbonate and pH (variable importance in projection > 1.5). Faeces and urine indicators were out of the top ten variables importance in projection.

Tables 5–7 give the models adapted to on-farm application and enriched to 3, 4 or 5 indicators giving performances in cross-validation equal or superior to the corresponding LDA non-enriched models. When adding blood haptoglobin (model 4C) to model 4 (blood bicarbonate – milk urea), the specificity and accuracy slightly increased compared to the initial model 4, with a marginal effect on sensitivity. When adding blood pH (model 4A), blood haptoglobin (model 4C) or milk SFA (model 4D) to model 4 (blood bicarbonate – milk urea), the specificity, precision and accuracy increased. When adding urine pH (4B2) to model 4B (blood bicarbonate – milk urea – blood BHBA), the precision increased compared to model 4B (88 vs 85%), without affecting the other parameters. When adding milk FPR to model 4C (model 4C1), precision and accuracy were improved (86 vs 81% and 83 vs 80%, respectively). Similarly, increases in sensitivity, specificity, precision and accuracy were observed by the addition of blood haptoglobin (model 5B) or faecal density (model 5C) to model 5 (blood bicarbonate + milk FPR). When adding diet forage (model 7A) or starch (model 7B) proportions, milk FPR (model 7C), blood urea (model 7D) or milk SFA (model 7E) to model 7 (blood BHBA

Table 5
Model performances of subacute ruminal acidosis detection in dairy cows using linear discriminant analysis combining controlled experiment (INRAE) and on-farm trial (FARM) data enriched with three indicators measurable by inexpensive routine analysis.

Model Villot et al. (2020)	Enriched model	Linear discriminant model variables						Model coefficients				Leave-one-out cross-validation performance of the models (%)[1]			
No.	No.	Matrix	Var 1	Matrix	Var 2	Matrix[2]	Var 3[2]	K	X1	X2	X3	Se	Sp	Pr	Ac
4	4A	Blood	HCO_3, mmol/L	Milk	Urea, mmol/L	Blood	pH	−42.9	0.206	0.543	4.65	77 ± 1.6	84 ± 1.0	84 ± 0.9	81 ± 1.0
	4B	Blood	HCO_3, mmol/L	Milk	Urea, mmol/L	Blood	BHBA, mmol/L	−8.83	0.212	0.434	1.27	77 ± 2.0	85 ± 1.2	85 ± 1.1	81 ± 1.2
	4C	Blood	HCO_3, mmol/L	Milk	Urea, mmol/L	Blood	Hp, log g/L	−9.30	0.221	0.489	−0.829	79 ± 1.1	81 ± 1.2	81 ± 1.4	80 ± 0.8
	4D	Blood	HCO_3, mmol/L	Milk	Urea, mmol/L	Milk	SFA, g/100 g FA	−14.1	0.205	0.628	0.082	76 ± 1.4	84 ± 1.1	83 ± 1.2	80 ± 1.0
5	5A	Blood	HCO_3, mmol/L	Milk	FPR	Diet	Starch, % DM	0.353	−0.115	−0.320	0.138	71 ± 1.5	81 ± 1.3	80 ± 1.4	76 ± 1.1
	5B	Blood	HCO_3, mmol/L	Milk	FPR	Blood	Hp, log g/L	−10.7	0.237	2.51	−0.975	73 ± 1.6	85 ± 1.0	82 ± 1.5	79 ± 0.9
	5C	Blood	HCO_3, mmol/L	Milk	FPR	Faeces	Density, kg/m^3	−4.95	0.242	2.37	−4.88	72 ± 1.1	85 ± 1.1	82 ± 1.4	78 ± 0.8
7	7A	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Diet	Forage, % DM	−8.11	−1.60	0.151	0.135	75 ± 1.6	95 ± 0.9	94 ± 0.9	84 ± 1.0
	7B	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Diet	Starch, % DM	−2.68	−0.272	−0.236	0.154	78 ± 1.6	86 ± 1.0	86 ± 0.8	82 ± 1.0
	7C	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Milk	FPR	−6.02	0.508	0.714	2.51	78 ± 1.6	83 ± 1.6	84 ± 1.3	81 ± 1.2
	7D	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Blood	Urea, mmol/L	−3.98	2.13	1.04	−0.351	81 ± 1.5	79 ± 1.7	81 ± 1.4	80 ± 1.3
	7E	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Milk	SFA, g/100 g FA	−10.8	0.704	0.829	0.105	79 ± 1.1	84 ± 1.5	84 ± 1.2	81 ± 1.1
10	10A	Milk	Urea, mmol/L	Urine	pH	Diet	Starch, % DM	0.237	−0.226	−0.395	0.154	80 ± 1.7	84 ± 0.9	86 ± 0.9	82 ± 1.1
	10B	Milk	Urea, mmol/L	Urine	pH	Milk	FPR	−19.8	0.738	1.84	1.68	77 ± 1.5	84 ± 0.9	85 ± 1.0	80 ± 1.0
14	14A	Faeces	pH	Milk	Urea, mmol/L	Diet	Starch, % DM	−0.479	−0.354	−0.220	0.149	77 ± 1.4	85 ± 0.9	84 ± 1.0	81 ± 1.0
	14B	Faeces	pH	Milk	Urea, mmol/L	Milk	SFA, g/100 g FA	−19.2	1.48	0.711	0.102	79 ± 1.3	86 ± 1.0	85 ± 1.1	83 ± 1.0

Abbreviations: BHBA = β-hydroxybutyrate; FA = fatty acids; FPR = fat/protein ratio; Hp = haptoglobin; K = constant; SFA = saturated FA; Var = variable; Xn = coefficient of variable n.
[1] Se = sensitivity; Sp = specificity; Pr = precision; Ac = accuracy; Mean from bootstrap procedure ± SE.
[2] Variable added to the corresponding model presented in Table 3.

Table 6
Model performances of subacute ruminal acidosis detection in dairy cows of using linear discriminant analysis combining controlled experiment (INRAE) and on-farm trial (FARM) data enriched with four indicators measurable by inexpensive routine analysis.

Model Villot et al. (2020)	Enriched model	Linear discriminant model variables								Model coefficients					Leave-one-out cross-validation performance of the models (%)[1]			
No.	No.	Matrix	Var 1	Matrix	Var 2	Matrix	Var 3	Matrix[2]	Var 4[2]	K	X1	X2	X3	X4	Se	Sp	Pr	Ac
4	4B1	Blood	HCO_3, mmol/L	Milk	Urea, mmol/L	Blood	BHBA, mmol/L	Milk	FPR	−9.53	0.200	0.424	0.890	1.21	78 ± 1.8	85 ± 1.6	86 ± 1.4	81 ± 1.2
	4B2	Blood	HCO_3, mmol/L	Milk	Urea, mmol/L	Blood	BHBA, mmol/L	Urine	pH	−10.0	0.203	0.446	1.29	0.178	78 ± 1.3	86 ± 1.3	88 ± 1.2	82 ± 1.1
	4C1	Blood	HCO_3, mmol/L	Milk	Urea, mmol/L	Blood	Hp, log g/L	Milk	FPR	−10.2	0.203	0.372	−0.848	1.69	81 ± 1.1	85 ± 1.3	86 ± 1.2	83 ± 0.8
5	5B1	Blood	HCO_3, mmol/L	Milk	FPR	Blood	Hp, log g/L	Faeces	Density, kg/m^3	−5.53	0.226	2.30	−0.950	−4.53	73 ± 1.8	84 ± 1.2	82 ± 1.5	79 ± 1.1
7	7B1	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Diet	Starch, % DM	Milk	FPR	−3.03	−0.222	−0.234	0.156	0.226	80 ± 1.7	85 ± 1.1	85 ± 1.0	82 ± 1.2
	7B2	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Diet	Starch, % DM	Urine	pH	−0.520	−0.279	−0.216	0.153	−0.286	79 ± 1.7	88 ± 1.0	89 ± 0.9	83 ± 1.2
	7B3	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Diet	Starch, % DM	Faeces	Density, kg/m^3	−3.52	−0.254	−0.230	0.153	0.828	79 ± 1.6	86 ± 0.9	86 ± 0.8	82 ± 1.1
	7C1	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Milk	FPR	Urine	pH	−19.2	0.917	0.691	1.66	1.76	79 ± 1.7	89 ± 1.3	90 ± 1.2	83 ± 1.4
	7D1	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Blood	Urea, mmol/L	Urine	pH	−16.2	2.24	1.01	−0.363	1.54	77 ± 1.6	85 ± 2.1	87 ± 1.7	81 ± 1.5
9	9A1	Blood	BHBA, mmol/L	Blood	Gluc, mmol/L	Urine	pH	Diet	Forage, % DM	−11.1	−0.857	4.20	−0.112	0.151	72 ± 1.6	96 ± 0.7	95 ± 0.8	83 ± 0.9
	9A2	Blood	BHBA, mmol/L	Blood	Gluc, mmol/L	Urine	pH	Milk	Urea, mmol/L	−18.1	1.64	0.640	1.69	0.771	77 ± 1.5	82 ± 2.2	84 ± 1.8	80 ± 1.7
10	10A	Milk	Urea, mmol/L	Urine	pH	Diet	Starch, % DM	Milk	FPR	1.53	−0.236	−0.637	0.157	0.539	81 ± 1.5	83 ± 1.1	86 ± 1.0	82 ± 1.2
	10A	Milk	Urea, mmol/L	Urine	pH	Diet	Starch, % DM	Blood	BHBA, mmol/L	−0.520	−0.216	−0.286	0.153	−0.279	79 ± 1.7	88 ± 1.0	89 ± 0.9	83 ± 1.2
	10A	Milk	Urea, mmol/L	Urine	pH	Diet	Starch, % DM	Milk	SFA, g/100 g FA	1.35	−0.250	−0.279	0.148	−0.027	80 ± 1.5	85 ± 0.9	87 ± 0.9	82 ± 1.1
	10B	Milk	Urea, mmol/L	Urine	pH	Milk	FPR	Blood	BHBA, mmol/L	−19.3	0.691	1.76	1.66	0.917	79 ± 1.7	89 ± 1.5	90 ± 1.3	83 ± 1.4

Abbreviations: BHBA = β-hydroxybutyrate; FA = fatty acids; FPR = fat/protein ratio; Gluc = Glucose; Hp = haptoglobin; K = constant; SFA = saturated FA; Var = variable; Xn = coefficient of variable n.
[1] Se = sensitivity; Sp = specificity; Pr = precision; Ac = accuracy; Mean from bootstrap procedure ± SE.
[2] Variable added to the corresponding model presented in Table 5.

Table 7
Model performances of subacute ruminal acidosis detection in dairy cows using linear discriminant analysis combining controlled experiment (INRAE) and on-farm trial (FARM) data enriched with five indicators measurable by inexpensive routine analysis.

Model Villot et al. (2020)	Enriched model	Linear discriminant model variables										Model coefficients						Leave-one-out cross-validation performance of the models (%)[1]			
No.	No.	Matrix	Var 1	Matrix	Var 2	Matrix	Var 3	Matrix	Var 4	Matrix[2]	Var 5[2]	K	X1	X2	X3	X4	X5	Se	Sp	Pr	Ac
7	7B2a	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Diet	Starch, % DM	Urine	pH	Faeces	Density, kg/m^3	−1.90	−0.256	−0.207	0.152	−0.247	1.03	80 ± 1.4	87 ± 1.1	89 ± 0.9	84 ± 1.1
	7C1a	Blood	BHBA, mmol/L	Milk	Urea, mmol/L	Milk	FPR	Urine	pH	Blood	Urea, mmol/L	−18.3	1.70	0.937	1.32	1.683	−0.346	77 ± 1.6	87 ± 3.1	88 ± 1.3	81 ± 1.4

Abbreviations: BHBA = β-hydroxybutyrate; FPR = fat/protein ratio; K = constant; Var = variable; Xn = coefficient of variable n.
[1] Se = sensitivity; Sp = specificity; Pr = precision; Ac = accuracy; Mean from bootstrap procedure ± SE.
[2] Variable added to the corresponding model presented in Table 6.

– milk urea), specificity increased from 66 to >78%, reaching 95% with diet forage proportion (in this case, however, to the detriment of sensitivity, which dropped from 79 to 75%). Similarly, precision and accuracy also increased. Three models originating from model 7 by adding a fourth indicator showed equivalent or slightly improved specificity and precision, although model 7D1 (model 7D urine pH) showed a lower sensitivity than model 7D. Only two models enriched with five indicators originating from model 7 maintained their discrimination, although without further improvement. The addition of diet forage proportion (model 9A1) or milk urea (model 9A2) increased specificity, precision, and accuracy, as well as sensitivity in model 9A1 (77 vs 69%). The addition of diet starch proportion (model 10A) or FPR (model 10B) to model 10 (milk urea – urine pH) improved specificity, precision and accuracy. Sensitivity was also higher in model 10A than in model 10 (80 vs 75%). Adding blood BHBA (10A2), to model 10A or adding blood BHBA (10B1) to model 10B resulted in slightly increased specificity, precision and accuracy. Finally, adding diet starch proportion (model 14A) or milk SFA (model 14B) to model 14 (faeces pH – milk urea) improved model specificity, precision and accuracy. The enrichment with a third and fourth indicator improved precision and accuracy for all the models, but this was not the case for the model enriched with five indicators.

Discussion

Evaluation of models developed by Villot et al. (2020) using commercial FARM data

When evaluating on-FARM data, the models built by Villot et al. (2020) in experimentally induced SARA, the discrimination capacity of the models decreased, meaning that their robustness was low. This low robustness can be due to a lack of homogeneity between the calibration and external evaluation datasets (Shenk and Westerhaus, 1995), as shown by their segregation in principle component analysis (Fig. 1), because of the FARM data were out of the validity domain of the models by Villot et al (2020). In the research by Villot et al. (2020), SARA was experimentally induced by a specific diet, and the SARA+ and SARA− groups were fed two contrasted diets (different forage/concentrate ratio, maize silage vs. grass silage, etc.). Such contrasting diets may have increased the amplitude of differences for some indicators (i.e. milk FA composition; faeces physical appearance/sieving, behaviour, etc. Cabiddu et al., 2022; Abdela, 2016; Humer et al., 2018). Occurrence of on-farm SARA in cows fed the same diet depends on the individual susceptibility of the cows (Khiaosa-ard et al., 2018). Furthermore, data collected on-farm usually cover intermediate situations to which the models developed in controlled trials are not usually confronted to. This means that the dataset on which the models of Villot et al. (2020) were calibrated for a specific range of response could be different from those from commercial farms.

Even if the indicators used by Villot et al. (2020) were fully biologically justified for the detection of SARA, another hypothesis to explain the low robustness of their models could be that the contrasting dietary conditions used in the experiment to induce SARA might have generated significant differences in the indicators, not primarily due to the occurrence of SARA. Some of the indicators in the models of Villot et al. (2020) might have been more sensitive to diet change than to SARA, like some FA (i.e. SFA, MUFA; PUFA, n-6 FA), which are greatly sensitive to cow feeding (Cabiddu et al., 2022). Discrimination models developed on the same indicators combining INRAE and FARM datasets gave both sensitivity and specificity superior to 70%. These results, as well as the principle component analysis (Fig. 2), seem to confirm our hypothesis that the low robustness could be due to heterogeneous calibration and external evaluation datasets, as these last data were out of the validity domain of the models by Villot et al. (2020).

Models development, adaptation and enrichment combining INRAE and FARM data

Model adaptation and enrichment combining INRAE and FARM data revealed new indicators relevant in discriminating between SARA+ and SARA− cows: blood haptoglobin, faecal density, and forage and starch proportions in the diet. Haptoglobin is a commonly reported inflammatory biomarker in plasma that is mainly involved in subacute or chronic inflammatory conditions (Horadagoda et al., 1999; Ametaj et al., 2011), and high plasma haptoglobin content has been associated with SARA (Zebeli et al., 2012). Changes in faecal density, with increasing frequency of diarrhoea, are observed in herds affected by SARA (Abdela, 2016). Low forage and high starch proportions in dairy cow diets are known to underlie SARA (Humer et al., 2018; Plaizier et al., 2018). On the other hand, the residue (5 + 2 mm) of sieved faecal material was not significant when including on-farm data. This could be due to the contrasted diets fed in the controlled trial by Villot et al. (2020) to induce SARA: their different fibre and grain contents led to a divergent distribution in sieving sizes (Abdela, 2016). When SARA occurs in cows fed the same diet (i.e. in a given farm), the distributions of sieved faecal material may be too close to discriminate between SARA+ and SARA− cows.

In general, when increasing the number of indicators per model from 2 to 3, the sensitivity in some cases and the specificity, precision and accuracy in almost all the models increased. Some increases in specificity and precision were observed when the models were enriched with 3–4 indicators. This confirms the hypothesis advanced by Plaizier et al. (2018), who reported that combining clinical examinations of cows including milk, blood urine and faeces indicators as well as diet characteristics would help to accurately detect SARA. However, the improvement was inconsistent when five indicators were used for the discrimination. Furthermore, the improvement in sensitivity and specificity was small when comparing models using four as opposed to three indicators and limited to only some of the models. Considering that sampling, analysis, and costs would be more consistent with a higher number of indicators, it would probably not be advisable to use more than three indicators for routine application of the models. The models developed in the present paper showed a potential for routine screening application as several of them had a sensitivity or a specificity close to or higher to the targeted threshold of 85%. However, at present, care in the application of our model for individual diagnosis of SARA is recommended as none of the models reached value >85% for both specificity and sensitivity, which can be considered a reasonable threshold to allow a possible use for on-farm screening (De Marchi et al., 2014; Villot et al., 2020; Coppa et al., 2021). A careful interpretation of the predicted results (i.e. by cross-checking the general health status of the animal) is also recommended since the indicators we used might also be sensitive to other diseases or to alteration in animal status (Abdela, 2016). Improvement of model reliability would probably be possible by enlarging the dataset, with more cows monitored on several different farms (for both NpH and indicators), with a view to increasing the variability of cow characteristics (i.e. including multiparous cows, different breeds, lactation stages, etc.) and reference data. Furthermore, external evaluation would also be needed.

In conclusion, the discrimination models developed by Villot et al. (2020) in controlled conditions were not robust enough when used in environments or timeframes different from those in which they were developed. Combining controlled experiment and on-

farm trial data and enriching each model from 2 to 3 indicators allowed to partially recover the discrimination capacity between SARA+ and SARA− cows, confirming the relevance and the potential of combinations of indicators measurable on-farm by inexpensive routine analysis for the detection of the risk of SARA at individual scale in a dairy herd. Therefore, enlargement of the datasets with more farms and cows i to develop the models, in order to increase variability and validity domain, is recommended to validate our results and further increase models performance in discriminating SARA. This would be probably preferable to adding further indicators to the models. Further research is needed to confirm this hypothesis. In addition, the use of models discriminating situations at risk of health problems is essential to reduce the number of experimental animals in line with the objective of the 3R approach (Refine, Reduce, Replace experimental animals; Vessier et al., 2021).

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100826.

Ethics approval

Both the experimental trial in controlled conditions and the on-farm trial were approved (No. C2E2A-02 8803-2017020115479420) by the French Ethics Committee.

Data and model availability statement

None of the data have been deposited in an official repository. The data that support the study findings are confidential.

Author ORCIDs

Mauro Coppa: https://orcid.org/0000-0003-2073-0599
Clothilde Villot: https://orcid.org/0000-0001-8955-3733
Cécile Martin: https://orcid.org/0000-0002-2265-2048
Mathieu Silberberg: https://orcid.org/0000-0003-3345-8088.

Author contributions

Mauro Coppa: conceptualisation, methodology, formal analysis, writing – original draft.
Clothilde Villot: conceptualisation, methodology, investigation, data curation, writing-review and editing.
Cécile Martin: conceptualisation, writing-review and editing, supervision.
Mathieu Silberberg: conceptualisation, methodology, writing-review and editing, supervision, project administration, funding acquisition.

Declaration of interest

None.

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