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Quantitative Analysis of the Relationship between Ruminal Redox Potential and pH in Dairy Cattle: Influence of Dietary Characteristics

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Abstract

The ruminal redox potential (E_h) can reflect the microbiological activity and dynamics of fermentation in the rumen. It might be an important indicator of rumen fermentation in combination with *pH*. However, the ruminal E_h has been rarely studied in dairy cows due to the difficulty of its measurement, and the relationship between ruminal E_h and *pH* is not clear. The objective of this study was to investigate the relationship between ruminal E_h and *pH* of dairy cows by meta-analysis of systematic measurements from different experiments. A database was constructed from 22 experiments on cannulated dairy cattle including 57 dietary treatments. The ruminal *pH* and E_h were measured without air contact between 0 and 8 h post-feeding. The results demonstrated a quadratic correlation between ruminal E_h and *pH* with a reliable within-animal variation ($E_h = -1697 + 540.7\ pH - 47.7\ pH^2$, $n_{observation} = 70$, $n_{animal} = 26$, $P < 0.001$, $RMSE = 56$, $AIC = 597$). The dietary characteristics (*NDF*, *NDFf*, *OM*, starch, degradable starch, soluble sugars contents, and the dietary ionic balance) influencing the ruminal *pH* also affected the ruminal E_h, but not always to the same extent. Some of them still influenced the relationship between ruminal E_h and *pH*. While the mechanism of the interaction between ruminal E_h and *pH* remains to be elucidated, it would be interesting to associate E_h to microbial profile, ruminal VFA concentration and milk production performance in future studies.

Keywords

Redox Potential, *pH*, Rumen, Diet, Dairy Cow

1. Introduction

Oxidation-reduction conditions are classically assessed by measuring the redox potential (E_h), also called oxidation-reduction potential (usually named ORP) expressed in millivolts (mV). It measures the ability of a solution to accept or donate electrons and corresponds to the potential difference between a platinum electrode and a standard hydrogen electrode [1]. Oxidation-reduction and acid-base reactions are essential for the maintenance of all living organisms. The chemistry of living organisms relies even more on oxidation-reduction reactions than it does on acid-base reactions, which are more focused on proton transfers [1] [2].

The role of E_h has been reported in many biological media such as dairy products [3], wine [4] and rumen fluid [5] [6] [7]. The ruminal E_h can reflect the microbiological activity and dynamics of fermentation in the rumen [8]. As a matter of fact, ruminal E_h is a mixed potential because of the strong fermentative activity involving numerous oxido-reduction couples. It reflects a weighted average of the potentials contributed by each of the redox couples as mentioned by De Laune and Reddy [9] for soil. The ruminal milieu is anaerobic with an E_h markedly negative, reflecting a strong reducing power in absence of oxygen [6]. It has been reported that dry matter intake can cause an increase of E_h, and the higher E_h also seems to be associated with higher concentrate proportions in the diet and lower ruminal *pH* [7], which may indicate digestive disorder. Indeed, a low E_h seems to be more favorable to the strict anaerobic bacteria such as fibrolytic and lactate utilizing bacteria [10]. Therefore, the ruminal E_h might be an important indicator of rumen function along with other ruminal variables. Until now, no threshold of ruminal E_h value has been proposed to evaluate rumen function. Since the ruminal pH is considered as the most direct indicator of the rumen digestive disorder and has been extensively studied [11] [12], comparing with ruminal pH could be helpful to interpret ruminal E_h value.

However, compared to other ruminal parameters, the E_h is rarely discussed in dairy cows, and the relationship between ruminal E_h and *pH* is not clear. Indeed, the ruminal E_h measurement method is not standardized. Three methods of E_h potentiometric measurements have been reported in the literature. The first one consisted of a manual suction-strainer device that pumped out ruminal fluid from a cannulated animal to measure E_h on collected hand-samples in contact with atmospheric air, after a stabilization period of 25 to 30 min as recommended by Andrade *et al.* [13] and adapted by Giger-Reverdin *et al.* [14]. The two others are *ex vivo* measurements performed on continuously pumped rumen fluid without air contact [6] and *in vivo* measurements performed conti-

nuously by wireless probes inside the rumen as described by Penner *et al.* [15] and adapted by Qin *et al.* [16]. Considerable difference in ruminal E_h values has been reported. The major difference is due to the different reference electrodes used. By definition, E_h is the potential difference between a platinum electrode and a standard hydrogen electrode. Some authors [13] [17] who used a reference electrode of calomel or silver chloride did not correct the raw E_h data (+199 mV at 39˚C). Also, the accurate ruminal E_h measurement requires strict anaerobic conditions which are not always satisfied [6].

For several years, our research team has conducted numerous experiments with simultaneous measurements of ruminal E_h and *pH* of dairy cows fed various diets under anaerobic conditions by *ex vivo* and *in vivo* methods. Analysis of these aggregated measurements could provide a better understanding of factors controlling ruminal E_h and *pH*, and might demonstrate a quantifiable relationship between ruminal E_h and *pH*. The objective of this study was to investigate the relationship between ruminal E_h and *pH* of dairy cows by meta-analysis of systematic measurements from different experiments.

2. Materials and Methods

2.1. Selection of Studies

A database was constructed from 22 experiments with cannulated dairy cattle including 57 dietary treatments (**Table 1**). As explained above, due to the heterogeneity of the ruminal E_h values reported in the literature, associated with time of measurement, anaerobic conditions and electrode used [5] [7] [8] [13] [14] [18] [19], we included in the database only experiments conducted by our research group and two others conducted in Agriculture and Agri-Food Canada (Research and Development Centre, Sherbrook, QC) to ensure a consistency of measurement methods among studies. It includes either published [7] [18] [20] [21] [22] and unpublished studies [23] [24]. Both lactating (12 experiments) and non-lactating cows (10 experiments) were used. Qualitative factors such as physiological status of animals (lactating *vs.* non-lactating) and site of the experiment (France *vs.* Canada) were collected.

All animal housing and handling procedures were in accordance with the guidelines for animal research of the French Ministry of Agriculture [25]. Cannulation techniques provided for humane treatment of cows, adhering to locally approved procedures, and were similar to those described by Streeter *et al.* [26]. All animals were housed in individual tie stalls throughout the experiment with free access to water. Each experimental period covered an adaptation period (2 to 3 weeks) to the different dietary treatment and a measurement period (3 days).

The diets were formulated to meet energy and protein requirements, with two equal distributions at 0900 and 1700 h. The composition of the diets (**Table 2**) varied widely (e.g. the proportion of concentrate ranged from 0 to 63%). Some of the dietary characteristics such as neutral detergent fiber from forages (***NDFf***), ruminally degradable starch, rumen protein balance (***RPB***) were estimated by the

Table 1. Summarize of 22 experiments in the database.

N_{exp}[1]	Physiological status	Experimental design	Method[2] for measuring E_h	Main ingredients of diets	Reference
1	Non-lactating	Latin square	1	Corn silage/wheat/corn/soybean meal	Unpublished
2	Lactating	Latin square	1	Corn silage/alfalfa hay/composed concentrate	[23]
3	Lactating	Randomized block	1	Corn silage/wheat/composed concentrate	Unpublished
4	Non-lactating	Latin square	1	Corn silage/wheat grain/corn/soybean meal	Unpublished
5	Non-lactating	Randomized block	1	Corn silage/alfalfa hay/corn/soybean meal	[21]
6	Non-lactating	Latin square	1	Grass hay/barley/wheat/soybean meal	[7]
7	Non-lactating	Randomized block	1	Alfalfa hay/corn silage/wheat straw/corn/soybean meal	[20]
8	Lactating	Latin square	1	Corn silage/wheat/soybean/meal/tanned soybean meal	[22]
9	Lactating	Latin square	1	Corn silage/wheat/corn/soybean meal	[24]
10	Lactating	Latin square	1	Corn silage/wheat/corn/soybean meal	[24]
11	Lactating	Latin square	2	Alfalfa silage/corn silage/grass hay/corn/soybean meal	Benchaar *et al.*, unpublished
12	Non-lactating	Randomized block	1	Corn silage/wheat/corn/soybean meal	Unpublished
13	Lactating	Latin square	2	Corn silage/alfalfa hay/soybean meal/composed concentrate	Unpublished
14	Non-lactating	Latin square	2	Grass hay/soybean meal	Unpublished
15	Lactating	Latin square	1	Grass hay/wheat/corn/soybean meal/composed concentrate	Unpublished
16	Non-lactating	Latin square	1	Corn silage/wheat/corn/soybean meal	Unpublished
17	Non-lactating	Latin square	1	Corn silage/wheat/corn/soybean meal	Unpublished
18	Lactating	Latin square	1	Corn silage/alfalfa hay/composed concentrate	[23]
19	Non-lactating	Randomized block	1	Corn silage/wheat/corn/soybean meal	Unpublished
20	Lactating	Latin square	2	Barley silage/corn silage/barley/corn/soybean meal	Benchaar *et al.*, unpublished
21	Lactating	Latin square	1	Corn silage/alfalfa hay/composed concentrate	[18]
22	Lactating	Latin square	1	Corn silage/wheat/composed concentrate	Unpublished

[1]N_{exp} = number of experiments; [2]Method 1 = measurements performed with probes on continuously pumped rumen fluid [6]; Method 2 = measurements performed continuously with probes inside the rumen and wireless device [15].

online software "systool.fr" [27] using the equations published in Sauvant and Nozière [28]. The influence of dietary ionic balance on acid-base balance of animal has been reported [29] [30] [31], it can be expressed (in mEq/kg of *DM*) as the dietary cation anion difference (***DCAD*** = Na + K-Cl-S) or electrolytic balance (***EB*** = Na + K − Cl). We also calculated these values according to the INRA tables [32] for all the diets used in the data base.

2.2. Measurement of Ruminal E_h and *pH*

A total of 775 kinetics of ruminal E_h and *pH* measurements were gathered together. Each kinetic includes 9 measurements of ruminal *pH* and E_h taken at 1 h

Table 2. Descriptive variables of the diets composition (n = 57) for data set used in the meta-analysis.

Item	Mean	*SD*	Minimum	Maximum
Intake, kg DM/cow per d	16.6	7.3	7.7	27.3
Proportion of concentrate, % *DM*	37.7	13.6	0.0	62.6
OM, g/kg *DM*	946.2	16.2	891.8	968.1
RPB, g/kg *DM*	4.0	17.8	−27.0	79.4
NDF, g/kg *DM*	368.5	73.8	263.3	566.3
NDFf, g/kg *DM*	303.0	92.3	178.5	566.3
Starch, g/kg *DM*	293.6	126.6	0.0	503.2
Degradable starch, g/kg *DM*	217.9	102.5	0.0	440.4
CP, g/kg DM	149.0	23.9	101.1	222.3
Soluble sugars, g/kg *DM*	50.6	28.4	0.0	105.4
DCAD, mEq/kg *DM*	173.3	99.3	59.1	438.0
EB, mEq/kg *DM*	276.9	119.5	133.8	638.0

DM = dry matter; *OM* = organic matter; *RPB* = rumen protein balance; *NDF* = neutral detergent fibre; *NDFf* = *NDF* from forages; *CP* = crude protein; *DCAD* = dietary cation anion difference (Na + K-Cl-S, in mEq/kg of DM); *EB* = electrolytic balance (Na + K − Cl, in mEq/kg of *DM*); *SD* = standard deviation.

intervals from the morning diet distribution to 8 hours after. The average E_h and *pH* of these 9 measurements have been calculated for each kinetic. The measurement of ruminal E_h and *pH* on each animal under each dietary treatment was repeated in three consecutive days during the measurement period.

All E_h and *pH* values were measured under strict anaerobic conditions, by *ex vivo* (Method 1) [6], or *in vivo* method (Method 2) [15]. In Method 1, rumen fluid was pumped continuously through a rubber tube into a 50-mL-double-walled thermo controlled vessel outside the rumen, the E_h and *pH* were measured by electrodes dipped in the collected rumen fluid without air contamination. In Method 2, a wireless real-time data logger (Dascor, Escondido, CA, USA) was submersed into the ventral rumen sac via the ruminal cannula after calibration, and the E_h and *pH* were measured by external sensors of the data logger and stored in the memory chip. For both methods, the accuracy E_h electrode was checked by measuring the standard solution at 220 mV (Fishier Scientific) before and after each measurement.

Considering both methods used an E_h platinum electrode, all records of the potential difference were corrected relative to the standard hydrogen electrode (+199 mV at 39˚C) [33]. Moreover, as Huang *et al.* [34] observed an effect of the method on the E_h value, due to the difference of sensors and location of measurements, the E_h values measured by Method 2 were corrected (+35.4 mV) to avoid the influence of method effect.

2.3. Statistical Analysis

Interpretation of the database was based on a statistical meta-analysis [35] [36]. At each step of the meta-analysis process, graphical observations were made to

check the coherence of relationships and to identify obviously abnormal values. All analyses were performed using the statistical software R version 2.15.1 (R Development Core Team, 2012).

2.3.1. Influence of Dietary Characteristics on E_h and *pH*

The average E_h and *pH* of each dietary treatment were calculated for this analysis. The experiment effect was considered to be random. The within-experiment correlation was calculated using a mixed model. The general form of the mixed model was:

$$Y_{ij} = B_0 + B_1 X_{ij} + s_i + b_i X_{ij} + e_{ij}$$

where i = number of studies, j = number of observations, $B_0 + B_1 X_{ij}$ is the fixed effect part of the model and $s_i + b_i X_{ij} + e_{ij}$ is the random effect part of the model. The goodness of fit of the model was evaluated using the Akaike Information Criterion (***AIC***) [37]. Because a reliable within-experiment response requires a minimal variation of descriptive variables, only the experiments tested a sufficient range of dietary characteristics (*OM* > 25 g/kg, starch > 70 g/kg, soluble sugar > 20 g/kg, *CP* > 18 g/kg, *NDF* > 80 g/kg, *DCAD* > 50 mEq/kg, *EB* > 100 m Eq/kg) were selected for within-experiment analysis.

For each relationship, the number of treatments (n_{treat}) and of experiments (n_{exp}) used in the analysis are reported. Treatments with high normalized residuals (<−3 or >+3) were identified and discarded from the model as statistical outliers if they had a high leverage effect based on *Hi* calculation (*Hi* > 3× k/n, where k is number of independent variables in the model and n is the number of observations) and Cook distance (Cook > 1) [35]. A one-way ANOVA was used to test whether ruminal E_h or *pH* varied according to the qualitative factors such as physiological status and site of the experiment.

2.3.2. Relationship between Ruminal E_h and *pH*

Since the individualized ruminal E_h and *pH* measurements are available, the average E_h and *pH* of each animal in each dietary treatment (3 repetitions) were calculated to take into account the variability within one animal under different dietary treatments. Only the animals (70 observations from 26 animals) presenting a sufficient range of ruminal *pH* (≥0.2) were selected to this analysis. The within-animal correlation was calculated using a mixed model. The animal effect was considered to be random. The model was:

$$Y_{ij} = B_0 + B_1 X_{ij} + s_i + b_i X_{ij} + e_{ij}$$

where i = number of animals, j = number of observations, $B_0 + B_1 X_{ij}$ is the fixed effect part of the model and $s_i + b_i X_{ij} + e_{ij}$ is the random effect part of the model.

The influence of co-variables (*OM*, *NDF*, *NDFf*, total starch, degradable starch, *CP*, soluble sugars, *DCAD*, *EB*, and *RPB* contents in diets) on the relationship between ruminal E_h and *pH* was tested. The first step consisted in highlighting the co-variables influencing the residuals (*i.e.* the difference between observed E_h and predicted E_h by the equation). The influence of all co-variableson residuals (observed minus predicted E_h) was tested using the Stepwise

procedure. In the second step of the analysis, the significant co-variables were included in the model.

3. Results

A summary of E_h and *pH* value in the database is given in Table 3. Both E_h (ranged from −233.4 to −99.6 mV) and *pH* (ranged from 5.48 to 6.76) covered a wide range.

3.1. Influence of Dietary Characteristics on Ruminal E_h and *pH*

Table 4 reports the relationship between ruminal E_h and dietary characteristics. Ruminal E_h was positively correlated to *OM* ($P = 0.022$), total starch ($P = 0.012$), degradable starch ($P = 0.041$), and soluble sugars ($P < 0.001$) contents, and negatively correlated to total *NDF* ($P = 0.024$), *NDFf* ($P = 0.049$), *DCAD* ($P < 0.001$), and *EB* ($P < 0.001$). The ruminal E_h was not related to *CP* ($P = 0.713$), and *RPB* ($P = 0.209$). No experiment tested the effect of intake and only two experiments tested a sufficient range of proportion of concentrate (≥30%), which did not permit the analysis of within-experiment relationship between ruminal E_h and these two parameters.

The quadratic adjustment was significant between ruminal E_h and *DCAD* ($E_h = -122 - 0.462DCAD + 0.000596DCAD^2$, $P = 0.010$, $RMSE = 9$, $AIC = 187$)

Table 3. Summary of the redox potential and *pH* value in the database.

	n	Mean	*SD*[1]	Minimum	Maximum
E_h (mV)	775	−179.8	25.9	−233.4	−99.6
pH	775	6.15	0.30	5.48	6.76

[1]*SD* = standard deviation.

Table 4. Relationship between ruminal redox potential and dietary characteristics.

Item	n_{exp}	n_{treat}	Intercept	Slope	*P*-value	*RMSE*	*AIC*
OM, g/kg *DM*	6	18	−718	0.559	0.022	13	151
RPB, g/kg *DM*	7	20	NS	NS	NS	NS	NS
NDF, g/kg *DM*	5	15	−143	−0.126	0.024	14	129
NDFf, g/kg *DM*	5	15	−165	−0.086	0.049	15	131
Starch, g/kg *DM*	6	18	−215	0.088	0.012	13	153
Degradable starch, g/kg *DM*	6	18	−210	0.089	0.041	14	155
CP, g/kg *DM*	6	18	NS	NS	NS	NS	NS
Soluble sugars, g/kg *DM*	6	18	−215	0.696	<0.001	10	137
DCAD, mEq/kg *DM*	8	22	−154	−0.145	<0.001	11	179
EB, mEq/kg *DM*	8	22	−141	−0.141	<0.001	12	174

OM = organic matter; *DM* = dry matter; *RPB* = rumen protein balance; *NDF* = neutral detergent fibre; *NDFf* = *NDF* from forages; *CP* = crude protein; *DCAD* = dietary cation anion difference (Na+K-Cl-S, in mEq/kg of *DM*); *EB* = electrolytic balance (Na + K − Cl , in mEq/kg of *DM*); n_{exp} = number of experiments; n_{treat} = number of treatments; *RMSE* = residual mean standard error; *AIC* = akaikeinformation criterion.

and between ruminal E_h and EB ($E_h = -107 - 0.368EB + 0.000313EB^2$, $P = 0.003$, $RMSE = 8$, $AIC = 183$). The ruminal E_h was significantly affected by physiological status (–188.5 ± 24.0 and –169.1 ± 20.8 mV for non-lactating and lactating cows respectively, $P = 0.002$), but not affected by the site of experiment ($P = 0.353$).

Table 5 reports the relationship between ruminal *pH* and dietary characteristics. Ruminal *pH* was positively correlated to *NDF* ($P = 0.008$), *NDFf* ($P = 0.012$), *DCAD* ($P = 0.004$), and *EB* ($P = 0.001$), and was negatively correlated to *OM* ($P = 0.018$), starch ($P = 0.004$), degradable starch ($P = 0.018$), and soluble sugars ($P < 0.001$) contents. It was not related to *CP* ($P = 0.195$) and *RPB* ($P = 0.518$).

No quadratic adjustment was significant for relationship between ruminal *pH* and dietary characteristics (data not shown). The ruminal *pH* was significantly affected by physiological status (6.32 ± 0.25 and 5.99 ± 0.17 for non-lactating and lactating cows respectively, $P < 0.001$), but not affected by the measurement method of E_h ($P = 0.942$), and the site of the experiment ($P = 0.950$).

3.2. Relationship between Ruminal E_h and *pH*

The relationship between ruminal E_h and *pH* is presented in Figure 1. The ruminal E_h and *pH* were negatively correlated. The linear relationship (Equation (1)) and quadratic adjustment (Equation (2)) were both significant ($P < 0.001$):

$$E_h = 104 - 46.3\,pH \left(n_{obs} = 70, n_{anim} = 26, RMSE = 17, AIC = 609\right) \quad (1)$$

$$E_h = -1697 + 540.7\,pH - 47.7\,pH^2 \left(n_{obs} = 70, n_{anim} = 26, RMSE = 16, AIC = 597\right) \quad (2)$$

3.3. Variables Influencing the Relationship between Ruminal E_h and *pH*

The intake ($P < 0.001$), soluble sugars contents ($P = 0.008$), *DCAD* ($P = 0.003$) were selected by the Stepwise analysis and significantly influenced the residuals

Table 5. Relationship between ruminal *pH* and dietary characteristics.

Item	n_{exp}	n_{treat}	Intercept	Slope	*P*-value	*RMSE*	*AIC*
OM, g/kg *DM*	6	18	10.93	−0.0049	0.018	0.11	2.1
RPB, g/kg *DM*	7	20	NS	NS	NS	NS	NS
NDF, g/kg *DM*	5	15	5.98	0.0011	0.008	0.10	3.5
NDFf, g/kg *DM*	5	15	6.14	0.0008	0.012	0.10	4.9
Starch, g/kg *DM*	6	18	6.57	−0.0008	0.004	0.10	2.9
Degradable starch, g/kg *DM*	6	18	6.52	−0.0008	0.018	0.11	5.4
CP, g/kg *DM*	6	18	NS	NS	NS	NS	NS
Soluble sugars, g/kg *DM*	6	18	6.54	−0.0055	<0.001	0.06	−14.3
DCAD, mEq/kg *DM*	8	22	6.05	0.0011	0.004	0.09	2.1
EB, mEq/kg *DM*	8	22	5.97	0.0010	0.001	0.11	5.5

OM = organic matter; *DM* = dry matter; *RPB* = rumen protein balance; *NDF* = neutral detergent fibre; *NDFf* = *NDF* from forages; *CP* = crude protein; *DCAD* = dietary cation anion difference (Na + K-Cl-S, in mEq/kg of DM); *EB* = electrolytic balance (Na + K − Cl , in mEq/kg of DM); n_{exp} = number of experiments; n_{treat} = number of treatments; *RMSE* = residual mean standard error; *AIC* = akaikeinformation criterion.

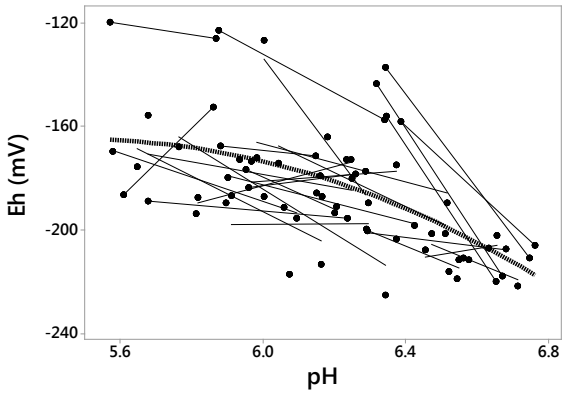

Figure 1. Relationship between ruminal redox potential (E_h) and *pH*. Each symbol represents the data from one animal in one experiment. The solid lines represent the linear regression of the data from each animal. The dotted line represents the average within-animal quadratic adjustment of all observations (E_h = −1697 + 540.7 *pH* −47.7 pH^2, $n_{observations}$ = 70, $n_{animals}$ = 26, P < 0.001, *RMSE* = 16, *AIC* = 597, R^2 = 0.77).

of Equation (2). Once included in Equation (2), only the *DMI* was significant (P = 0.03) and slightly improved the equation:

$$E_h = -2097 + 690.2\,pH - 60.7\,pH^2 - 1.27\,DMI$$
$$\left(n_{obs} = 70, n_{anim} = 26, RMSE = 16, AIC = 591\right) \quad (3)$$

4. Discussion

Meta-analyses use scientific methods based on statistics to summarize and quantify knowledge acquired through previously conducted studies [35]. Until now, there is alimited number of studies reporting ruminal E_h measurements. Unlike a classical empirical modeling of biological responses based on exhaustive data collection from published experimental results, our study used the aggregation of measurements from our experiments in order to ensure the homogeneity of E_h values and avoided the considerable influence of measurement method explained previously. Use of such analysis leads to a better understanding of factors that controlling the variables.

The database of present study covered a wide range of ruminal E_h and *pH* values. The range of ruminal E_h value in dairy cattle in our database (−233.4 to −99.6 mV) is comparable with that in sheep (−260 to −150 mV) [8] [19], in goat (−190 to −145 mV) [5] and in dairy cow (−241 to −185 mV) [38]. Some authors reported much lower ruminal E_h values: from −340 to −302 mV in sheep [17] and from −352 to −327 mV in goat [13]. It is due to the different reference electrodes used as explained above. The significant effect of physiological status on ruminal E_h and *pH* was expected and could be explained by dietary difference between lactating and non-lactating cows.

4.1. Dietary Characteristics Influencing Ruminal E_h

The influence of dietary concentrate proportion on ruminal E_h observed in previous studies [5] [8] [14] was not confirmed by our analysis due to the limited

number of experiments (n = 2) presenting a sufficient range of dietary concentrate proportion. However, the variables associated with slowly or rapidly degradable materials contents (*NDF*, *NDFf*, *OM*, starch, degradable starch and especially soluble sugars, which resulted low *RMSE* and *AIC*) showed consistent correlation with ruminal E_h.

Few studies investigated the influence of these dietary characteristics on ruminal E_h. However, the effect of slowly or rapidly degradable diet on ruminal E_h has been reported. Andrade *et al.* [13] observed a higher ruminal E_h for the goats fed rapidly degradable diet (−327 mV) compared to that of goats fed slowly degradable diet (−352 mV). These E_h values were lower than ours due to the different reference electrodes used, but the difference of ruminal E_h caused by two type of diet was significant ($P < 0.001$). Our results are in agreement with these observations.

To our knowledge, the effect of dietary ionic balance (*DCAD* and *EB*) on ruminal E_h has never been reported. According to our results, the *DCAD* and *EB* showed consistent correlation with ruminal E_h. The quadratic adjustment of the within-experiment relationship resulted slightly higher *AIC* (187 and 183 for *DCAD* and *EB* respectively) but lower *RSME* (9 and 8 for *DCAD* and *EB* respectively). The mechanism of this effect remains unclear. But it is known that E_h can affect mineral availability. As demonstrated in soil, E_h is a factor that strongly influences the mobility of many elements such as N, P, S, K and Na. Conversely, E_h is influenced by the various elements [1]. Considering that the effect of dietary ionic balance was not investigated as a determining factor by the experiments in the database, it deserves to be confirmed by a classic experiment with *in vivo* measurements.

4.2. Dietary Characteristics Influencing Ruminal *pH*

The influence of *OM*, *NDF*, *NDFf*, starch, degradable starch and soluble sugars contents on ruminal *pH* is well documented. Among these variables, the relationship between *NDF* and starch content and ruminal *pH* are frequently studied. The relationship ($y = 5.53 + 0.022x$) between *pH* and diet *NDF* content (% *DM*) reported by Pitt *et al.* [39] is close to the relationship obtained in our study. By analyzing results from 23 studies of lactating dairy cows fed pasture, Kolver and de Veth [40] reported a within study equation between ruminal *pH* and *NDF* content (% *DM*) with a numerically lower slope than ours ($y = 5.84 + 0.0075x$, $P = 0.014$, $n = 100$), when taking into account the difference of unit of *NDF* (g/kg *DM* in our analysis). Regarding the influence of degradable starch in the rumen (% of intake dry matter) on ruminal *pH* (dairy and beef cattle), Sauvant and Peyraud [11] reported a similar relationship ($y = 6.4 - 0.01x$) compared to ours.

The *DCAD* and *EB* are close (the only difference is that the *EB* does not consider sulfur ions) and highly correlated [41]. Both influence ruminal *pH*. Their influence on acid-base balance of animal has been described [42]. Indeed, Na and K are absorbed from the gastrointestinal tract in exchange for the secretion

of a proton, whereas Cl and S are often absorbed in exchange for the secretion of a bicarbonate ion [31] [43]. Increasing *DCAD* in the diet allows the cows to overcome the saturation of the renal mechanisms for saving HCO_3 and contributes to increase blood bicarbonate concentration which could be recycled into the rumen to limit the decrease of ruminal *pH*. Several studies reported that a shift from negative or null *DCAD* to highly positive values increases *DMI* and milk yield [42] [44]. A meta-analysis [30] grouping 27 experiments reported positive relationship between *EB* and blood *pH*, *EB* and bicarbonate content in blood, *EB* and *pH* of urine. Our results showed clear positive relationship between *DCAD* or *EB* and ruminal *pH*, which is in agreement with the hypothesis of the acid-base balance mechanism in ruminant. The equation between ruminal *pH* and *DCAD* obtained by our analysis is consistent with that of Iwaniuk and Erdman [45], obtained by a meta-analysis of 63 published journal articles (y = 6.31 + 0.0003x, P = 0.034, r^2 = 0.19, n = 83). Considering these results, *DCAD* and *EB* deserve to be more often measured and taken into account in future studies.

4.3. Relationship between Ruminal E_h and *pH*

The results of present study confirmed the negative relationship between ruminal E_h and *pH* reported by previous studies in goats [5] [13] [46]. The slope of the linear relationship in our study is similar to that of Giger-Reverdin *et al.* [46]. The lower average ruminal E_h value (−354 ± 22 mV) reported by these authors could be explained by the different measurement methods used as explained previously. By gathering together a large data base of wide range ruminal E_h and *pH* values, we further demonstrated a quadratic correlation Equation (2) between ruminal E_h and *pH* with a reliable within-animal variation of the variable. Considering that in biological media, such as rumen, many oxidation-reduction reactions involve protons, it is not surprising that ruminal E_h and *pH* are related [1] [13] as is shown by the Nernst's equation [47].

It is noteworthy that the diet characteristics (*NDF*, *NDFf*, *OM*, starch, degradable starch, soluble sugars contents, and the dietary ionic balance) influencing the ruminal *pH* also affected ruminal E_h, but not always in same extent. Indeed, the complex reactions which determine E_h are not necessarily the same reactions which determine *pH*: for example, when rapidly-oxidizable organic matter is added, the E_h could be changed without changing *pH* [48]. Also, Friedman *et al.* [49] highlighted the E_h as a key factor in the structuring of anaerobic microbial communities through their experimental system separating E_h from *pH* effect.

In our database, we can observe some high *pH* values (e.g. *pH* > 6, without SARA according to the ruminal *pH* thresholds proposed in the literature) associated with high E_h which is unfavorable to activities of fibrolytic and lactate utilizing bacteria, and also some low E_h values associated with low *pH* (**Figure 1**). Therefore, in some circumstances, the E_h could better reflect the fermentation dynamics than *pH* and *vice versa.*

The measurement of ruminal *pH* alone might not be sufficient for diagnosing digestive disorder in some cases. The simultaneous measurement of ruminal E_h and *pH* could be useful to provide complementary information about the rumen fermentation. Nevertheless, no threshold has been proposed to evaluate the rumen digestive disorder. In order to initiate the use of ruminal E_h, we could propose a preliminary threshold of ruminal $E_h > -166$ mV (correspond to $pH < 6$ according to Equation (2)) indicating digestive disorder.

5. Conclusion

By gathering together a large database of uniformly measured ruminal E_h and *pH* under anaerobic conditions, the present study demonstrated a quadratic correlation between ruminal E_h and *pH*. The analysis highlights the influence of dietary characteristics on ruminal E_h. Within experiments, a good prediction of ruminal E_h could be made using soluble sugars content and the dietary ionic balance. The dietary characteristics (*NDF*, *NDFf*, *OM*, starch, degradable starch, soluble sugars contents, and the dietary ionic balance) influencing the ruminal *pH* also affected the ruminal E_h, but not always in same extent. Some of them still influence the relationship between ruminal E_h and *pH*. The mechanism of the interaction between ruminal E_h and *pH* remains to be elucidated; it would be interesting to associate microbial profile and ruminal VFA concentration and milk production performance in future studies.

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