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Individual Variability in Susceptibility to the Occurrence of Low Rumen pH in Lactating Dairy Cows: What Has Been Done and What Needs to be Done

Review Article

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ABSTRACT

Individual variability in susceptibility to low rumen pH is one of the currently known aspects of rumen acidosis in dairy cows, and its understanding and management may address part of the complications of rumen acidosis. This review aims to summarize the outputs of current findings on individual variability in susceptibility to low rumen pH in terms of causes and effects and to provide strategies to manage it in research and commercial production systems. On the basis of the present findings, variability in rumen pH is detected among dairy cows with the same dietary and management conditions, and sometimes, the rumen pH can differ more than that associated with any dietary intervention. Cows with a high risk of low rumen pH are characterized by greater sorting against long particles in their diet; lower body weight, feed intake and milk fat; and longer rumination times. However, the mechanisms of these changes are not strong enough to cover the variation in the rumen pH of animals fed the same diets, and more research is needed to evaluate the mechanisms involved. As the variability in rumen pH can affect the results of other experiments at the level of research infrastructure and production efficiency at the level of commercial farms, a precise evaluation needs to be conducted to determine the key elements both biologically and quantitatively. Such biological and quantitative evaluation opens perspectives for managing this problem at both levels of research infrastructure and commercial farms. Pioneering work has started to address individual variability at the commercial level, including grouping strategies, animal selection, and increasing feeding frequency. However, this early evidence is not enough to make comprehensive recommendations, and these concepts need to be studied thoroughly. For research experiments, it is also suggested to measure the rumen pH at the beginning of each experiment to use it as a blocking factor or covariate, but this is also the initial level. In conclusion, individual variability in susceptibility to low rumen pH is a common and determining phenomenon among dairy cows in both experimental and commercial production systems but is not well understood in terms of causes and effects and is rarely known in terms of handling and management.

KEY WORDS dairy cows, individual variability, review rumen acidosis.

INTRODUCTION

Rumen acidosis has been a prevalent disorder in ruminants for a long time, and researchers have been trying to develop strategies to prevent this disorder. Despite decades of research, this syndrome is still prevalent, and disrupting the health, production, and well-being of animals (Kitkas *et al.* 2013; Kleen *et al.* 2013; Stefańska *et al.* 2017; Huot *et al.* 2023; Golder and Lean, 2024) results in enormous economic losses (Rojo-Gimeno *et al.* 2018; Srivastava *et al.* 2021). A recent study reported an average true prevalence of 16% of the herd, which induced a cost of disease of

€210/case/year and a cost of treatment of €20 - €250/case/year Rojo-Gimeno *et al.* (2018). There are still considerable gaps in knowledge around the concept of rumen acidosis, and the current knowledge needs to be discussed to open new windows and viewpoints to understand this phenomenon more clearly (Plaizier *et al.* 2018; Sun *et al.* 2018; Laporte-Uribe, 2019; Coppa *et al.* 2023).

Recent studies have indicated tremendous individual variability in susceptibility to the occurrence of low rumen pH (Gao and Oba, 2014; Nasrollahi *et al.* 2017; Yang *et al.* 2022; Zhang *et al.* 2023; Hartinger *et al.* 2024), even at the farm level (Huot *et al.* 2023).

Indeed, it has been demonstrated that animals with the same dietary conditions show considerable variation in rumen pH, resulting in distinctive categories (i.e., high-risk *vs*. low-risk animals). For example, Gao and Oba (2014) reported that in high-risk *vs*. low-risk animals, the pH was less than 5.8 in 556 *vs*. 10 min/d, the pH was less than 5.8 in 9.92 *vs*. 0.86 Ph × min, and the acidosis index (pH less than 5.8/DMI) was 7.67 *vs*. 0.037 pH × min/kg DMI. Nasrollahi *et al.* (2017) measured reticular pH with boluses and reported that for high-risk *vs*. low-risk animals, the aforementioned values were 920 *vs*. 78 min, 379 *vs*. 40 pH × min, and 15.5 *vs*. 2.48 pH × min/kg DMI, respectively. This difference between individuals was even greater than that of experimentally induced low rumen pH; For example, Li *et al.* (2012) reported that the time and area of rumen pH below 5.8 for low rumen pH induced by feeding alfalfa hay pellets were 489 min and 107 pH × min, respectively, and for low rumen pH induced by feeding grain pellets, the values were 514 min and 133 pH × min, respectively, whereas those for the control diet were 188 min and 28 pH × min, respectively.

Since the beginning of the previous decade, researchers have tried to document factors related to this individual variability, and several factors have been proposed. At the same time, several researchers have tried to evaluate approaches to manage or exploit this condition. Despite interesting review papers that have recently been published on other aspects of rumen acidosis (Fu *et al.* 2022; Plaizier *et al.* 2022; Golder and Lean, 2024), the concept of individuals variably has not been well reviewed, and there is a gap in summarizing, comparting and integrating the current knowledge on this subject.

Therefore, the objective of this review is to summarize findings on the individual variability in the rumen pH of dairy cows and thereby provide some perspectives for managing it in both research infrastructure and industrial production systems.

Factors related to individual variability in susceptibility to the occurrence of low rumen pH

Feeding behavior, feed intake, and body weight

Feeding behavior is a well-known way to develop diets-related low rumen pH (DeVries *et al.* 2008; Nasrollahi *et al.* 2014; Coon *et al.* 2019). Cows sorting diets against long and fiber particles are prone to develop low rumen pH first by lowering the chewing time per kg of diet (Beauchemin, 2018) and second by ingesting low-fiber and high-fermentable particles (DeVries *et al.* 2008; Nasrollahi *et al.* 2021). Lowering the chewing time causes lower saliva excretion, and a higher fermentation rate augments the rumen VFA and acid load, both of which result in a decrease in the rumen pH (Allen 1997; Beauchemin, 2018). Gao and Oba (2014) reported that high-risk cows tend to select short particles, which was confirmed in another study with high milk-producing cows where high-risk cows selected against long particles early after feeding (Nasrollahi *et al.* 2017). A clear effect of sorting against long particles in high-risk animals has been reported by Coon *et al.* (2019), where only in high-risk animals did switching from short-cut forage to long-cut forage cause sorting against long particles (particles retained on 19 and 8 mm of PSPS). Therefore, high-risk animals are fine-particle selectors, and the selection of these fine particles by high-risk animals may explain part of the low rumen pH results.

In almost all available studies conducted on individual variability in susceptibility to the occurrence of low rumen pH depending on the number of animals used, there was a significant or numerically greater body weight and feed intake for low-risk animals than for high-risk animals in the midlactation stage. Nasrollahi *et al.* (2017), with individual monitoring of 78 animals, reported that low-risk animals weighed 53 kg more and ate 1.9 kg DM/d more feed, whereas Gao and Oba (2014), using 16 animals, reported that low-risk *vs*. high-risk animals had a numerically greater body weight (57 kg) and feed intake (1.8 kg DM/d). The trend for DMI has also been recently observed in transition cows (from 1-week prepartum to 3-week postpartum periods) (Yang *et al.* 2022). It was demonstrated that this high feed intake was for large cows that intended to eat long fiber particles and that rumen health would be guaranteed (Nasrollahi *et al.* 2017).

On the basis of background knowledge of the relationship between chewing activity and rumen pH, low-risk animals are expected to have greater rumination times than high-risk animals. Additionally, the previously mentioned ability involves the selection of coarser particles by low-risk animals, which are prone to more chowing activity than are high-risk animals. However, research has shown that high-risk animals have a greater rumination time per kg of DMI (Gao and Oba, 2014; Nasrollahi 2017; Khiaosa-ard *et al.* 2018). Researchers explain this phenomenon as an effect and not a cause of low rumen pH in high-risk animals. Indeed, because high-risk animals suffer from low rumen pH, an adaptive response can be achieved by increasing buffer secretion with increasing rumination time (Gao and Oba, 2014). Additionally, the low rumen pH in high-risk animals can cause lower fiber digestion, and retaining such fiber particles in the rumen can motivate more rumination (Gao and Oba, 2014). Greater rumination activity in animals with lower rumen pH has also been recently documented in male Hu sheep (Zhang *et al.* 2023).

There is some evidence that conditions are more complex than these explanations are. It has been shown that dietary modification increases rumination activity and salivary secretion, but there is likely no parallel increase in buffer secretion (Bailey and Balch, 1961; Storm *et al.* 2013). Moreover, because increased saliva production during chewing may decrease saliva production during rest, the net saliva or buffer added by increased chewing activity may be minimal (Maekawa *et al.* 2002; Jiang *et al.* 2017; Beauchemin, 2018). If the net amount of saliva or buffer does not increase enough, increasing rumination can even negatively influence the rumen pH because it decreases the particle size and increases the digestion rate (Beauchemin, 2018); as a result, the rumen pH decreases. Research that has attempted to evaluate rumination time across different times of the day between high-risk and low-risk animals has indicated that a greater rumination time in high-risk animals occurs at nighttime of the day, and this rumination is associated with a greater time and area of rumen pH below 5.8 (Macmillan *et al.* 2017). To gain clear insight into rumination and its related role in susceptibility to the occurrence of low rumen pH, the amount of saliva produced and its content of buffers and the coincidence of the time of acid production and buffer secretion should be measured.

Rumen microbiome, VFA production, and absorption

Most of the research conducted on the rumen microbial community did not find any relationship between individual variability in rumen pH and the rumen microbial population (Mohammed *et al.* 2012; Gao and Oba, 2016; Zhang *et al.* 2020). Mohammed *et al.* (2012) conducted a study on microbial populations in transition dairy cows fed a normal or high-concentrate diet before calving. Although they observed individual variability in both the rumen pH and the microbial population, the variation in these two elements was not related. Zhang *et al.* (2020) also reported a variation in cellulolytic bacteria among fattening lambs fed a high-concentrate diet, which was correlated with rumen VFA but not pH. In another study, the activity of the rumen bacteria of high-risk and low-risk cows was evaluated via *in situ* incubation of starch and NDF, but high-risk and low-risk animals experienced similar amounts of degradation (Gao and Oba, 2016). A recent study revealed the abundance of some unidentified rumen species (Spirochaetaceae, Anaeroplasma, *Fusarium_oxysporum*, and *Papiliotrema_laurentii*) that discriminate high-risk cows from low-risk cows (Hu *et al.* 2021). Additionally, a more recent study reported a decrease in the copy number of *Fibrobacter succinogenes* in the rumen, as well as in the digestibility of NDF and ADF in high-risk *vs.* low-risk sheep (Zhang *et al.* 2023). Since the anaerobic microbial ecosystem of the rumen has a very complex nature, it is possible that some indicative elements within the bacterial community were not detectable with available devices and approaches (Krause *et al.* 2013; Choudhury *et al.* 2015; Hu *et al.* 2021). Therefore, more research is needed to address whether the rumen microbiome is indicative of susceptibility to the occurrence of low rumen pH.

Similarly, with respect to rumen bacteria, there are limited differences in VFA concentration and composition between high-risk and low-risk animals (Gao and Oba, 2014; Nasrollahi *et al.* 2017; Khiaosa-ard *et al.* 2018). Only when animals were force-fed a high-concentrate diet (Schlau *et al.* 2012) or a glucose drench (Penner *et al.* 2009) did the total VFA or acetate proportion differ, and high-risk animals presented greater VFA (Schlau *et al.* 2012) and lower acetate (Penner *et al.* 2009) concentrations. In research on the regular feeding conditions of middle- or late-lactating Holstein cows, VFA compositions were not affected, and some minor changes occurred in some rare VFAs, such as valerate, isovalerate, or isobutyrate (Gao and Oba, 2014; Nasrollahi *et al.* 2017); however, in these studies, the effects were not consistent. The authors also indicated lower genetic variability in lactating Holstein cows than in beef steers or sheep as another reason for the observed discrepancy between the results. Valerate in the study of Nasrollahi *et al.* (2017) related to the rumen pH (-), blood concentration of aspartate aminotransferase (+), and milk fat percentage (-) (data reported in Nasrollahi (2017)), and further research reported a strong negative correlation coefficient between valerate and cellulolytic bacteria in the rumen (Zhang *et al.* 2020). Recently, it was reported that in transition cows, ruminal propionate proportions are greater in high-risk than in low-risk animals, which may indicate that the stage of lactation may affect the variation in VFA production in the rumen (Hartinger *et al.* 2024).

Apical uptake of acetate (7.4 *vs.* 3.2 nmol/mg protein/min) and butyrate (13.7 *vs.* 8.8 nmol/mg protein/min) by rumen epithelial cells was significantly greater for low-risk sheep than for high-risk sheep, which caused a greater b-hydroxybutyrate concentration in the plasma (Penner *et al.* 2009). However, bicarbonate-independent uptake differed between low-risk and high-risk animals. Because bicarbonate-independent uptake is responsible for transporting the undissociated form of VFA (VFA-H), a high gradient of H^+ is expected at the intracellular space of the rumen epithelial cells of low-risk animals, which can potentially adversely affect the activity of these cells (Aschenbach *et al.* 2011). Further work on the epithelial cells of steers revealed that the mRNA abundance of sodium/hydrogen exchanger isoform 3 in ruminal epithelial cells was greater for low-risk steers than for high-risk steers (Schlau *et al.* 2012). However, as previously mentioned, both Penner *et al.* (2009) and Schlau *et al.* (2012) conducted extreme experiments to produce animals with extremely low and high rumen pH, and VFA absorption was affected. In a further study of lactating dairy cows, no relationship was observed between susceptibility to low rumen pH and the mRNA abundance of sodium/hydrogen exchangers (Gao and Oba, 2016). This may be related to the lower severity of the low rumen pH challenge and the low diversity of the Holstein cows used. From the results of the rumen microbial community and epithelial capacity for VFA uptake, it can be concluded that, at least with current *in vitro* techniques, there is no detectable difference between animals showing different rumen pH values when they are fed a practical high-concentrate diet.

Blood metabolism

Circulating blood is a pool of body metabolisms facing almost all body organs and is therefore an appropriate source for evaluating the metabolic conditions of low-risk and high-risk animals. Considering the concept of individual animal variability in rumen pH, body metabolites can potentially be related to causes or effects. Some metabolites can affect saliva production (Carter and Grovum, 1990), behavior (Williams and Elmquist, 2012), or rumen motility (Ingvartsen and Andersen, 2000) and cause a low rumen pH. On the other hand, changes in rumen metabolites and their absorption during periods of low rumen pH can affect body metabolism and blood circulation properties (Minuti *et al.* 2014; Khiaosa-ard *et al.* 2018; Nasrollahi *et al.* 2019). Therefore, separating the causes or effects of blood metabolism on individual variability in susceptibility to the occurrence of low rumen pH is difficult.

One of the metabolites that is supposedly susceptible to the occurrence of low rumen pH is beta-hydroxybutyric acid (BHBA), which is the output of the rumen epithelial metabolism of the absorbed acetate and butyrate.

As mentioned, the epithelial capacity for apical uptake of these two fatty acids is greater in low-risk than in high-risk animals (Penner *et al.* 2009), resulting in a greater BHBA concentration in the plasma (Penner *et al.* 2009). This trend was similar to the numerical trends reported in most other studies (Schlau *et al.* 2012; Khiaosa-ard *et al.* 2018; Nasrollahi *et al.* 2019) (P=0.08-0.14). However, some studies did not observe such a difference in BHBA between high-risk and low-risk animals, but they reported greater mRNA levels of enzymes effective in cholesterol synthesis (Gao and Oba, 2016). As confirmed by Khiaosa-ard *et al.* (2018), cholesterol synthesis was a possible alternative metabolic pathway in the apical cells of the rumen in those studies.

The change in the urea-N concentration in the blood or milk has also been suggested as the effect of individual variability in the rumen pH, but the relationship of susceptibility to the occurrence of low rumen pH and urea-N concentration has been inconsistent, varying from negative (Gao and Oba, 2014) to nonsignificant (Khiaosa-ard *et al.* 2018) to positive (Nasrollahi *et al.* 2019; Stefańska *et al.* 2020). Nasrollahi *et al.* (2019) proposed that the state of lactation might be an interfering factor and that the relationship in late-lactation cows is negative and that in mid-lactation cows is positive, but it is not clear which exact biological reason explains the discrepancy among different stages of lactation.

The concentration of aspartate aminotransferase has recently been suggested as an indicator of discrimination between high-risk and low-risk animals (Nasrollahi *et al.* 2019), but again, this result was not confirmed by other studies (Khiaosa-ard *et al.* 2018; Hartinger *et al.* 2024). The relationship between blood metabolites and individual variability is highly experimentally dependent, which negatively affects the repeatability of the results.

Milk fat content

Milk fat depression is one of the known side effects of diet-mediated low rumen pH, and several mechanisms have been suggested (NRC, 2001). Since most of these hypotheses have been built on the basis of low rumen pH, it could be hypothesized that cows that are at high risk of having a low rumen pH would also have a lower milk fat content. This perception has been validated in most of the studies on mid-lactation cows (Nasrollahi *et al.* 2017; Khiaosa-ard *et al.* 2018). Those studies that did not detect a significant difference still reported a high numerical difference (Gao and Oba, 2014; Gao and Oba, 2016), and it is possible that not detecting a significant effect might be due to low statistical power related to the low numbers of animals used.

The results were generally the same for fat percentage and yield, but the intensity of the difference in fat percentage was greater than that of fat yield (Nasrollahi *et al.* 2017; Khiaosa-ard *et al.* 2018).

A further study that aimed to correlate milk fat content with the composition of milk fatty acids indicated that cows with high amounts of C18:1 trans-10 fatty acids were more susceptible to low rumen pH (Jing *et al.* 2018). In this study, cows with high amounts of C18:1 trans-10 had lower minimum and mean rumen pH values and greater times with pH values less than 6.0 and tended to have greater areas with pH values less than 6.0 and acidosis indices (areas with pH values less than 6.0/DMI). However, the results not consistent for all cows [a limited number of animals with high C18:1 trans-10 ratios had high rumen pH values (one out of five) and vice versa], and no relationship between C18:1 trans-10 ratios and pH values less than 5.8 or 5.6 was observed. In this study, cows with high amounts of C18:1 trans-10 fatty acids presented elevated levels of C15:0 and a sharp decrease in C18:1 trans-11. The cows with high C18:1 trans-10 ratios had a lower milk fat-to-protein ratio and tended to have a greater milk fat content (P=0.08) despite the small number of animals used (n=5). This effect of C18:1 trans-10 is related to its biological role in the downregulation of milk fat synthesis in mammary glands (Bauman and Griinari, 2001). Therefore, the C18:1 trans-10 produced in the rumen has a possible role in the low milk fat content of animals, which is associated with a high risk of low rumen pH.

Summing up

A summary of the characteristics of cows with a relatively high susceptibility to low rumen pH is shown in Figure 1. Cows with a high incidence of low rumen pH are characterized by increased sorting of long particles in the diet; decreased body weight, feed intake and milk fat; and increased rumination time. The results concerning the rumen environment and blood metabolites indicate that some inconsistent effects on lower rumen apical absorption of VFAs and greater concentrations of some types of blood metabolism in these cows need more investigation. Overall, these suggested mechanisms are not strong enough to cover the large variation in the rumen pH of animals fed the same diets, and more research is needed to evaluate the underlying mechanisms.

Differences in low rumen pH due to individual differences and dietary conditions

One important question about individual variability in susceptibility to low rumen pH occurrence is whether the decrease in rumen pH is the same when the cause is animal variability (low-risk *vs*. high-risk animals) or a dietary effect. We compared the results of some studies from these two categories, which are reported in Table 1. When low rumen pH is diet-induced, compared with low rumen pH due to individual variability, a massive response of increased inflammatory biomarkers and rumen VFA concentrations as well as a more pronounced decrease in milk fat concentration are observed. Surprisingly, in this comparison, the amount of reduced pH due to individual variability (i.e., difference between high-risk and low-risk animals= -629 min/d on average) was much greater than that due to diet induction (i.e., difference between the control and induced diets= -352 min/d on average). It is evident that low rumen pH due to individual variability is completely different from low rumen pH due to diet induction. It can be hypothesized that cows that are inherently at high risk of having a low rumen pH after several generations become institutionalized in that even with a low rumen pH, they can manage their health and production more or less, and these cows are not necessarily ill despite having a low rumen pH. Another explanation for this observation could be the role of dCO_2 and CO_2 holdup, which have not been measured in these studies, and as previously explained, a change in ruminal pH may not necessarily cause disease if not accompanied by a change in CO_2 species (Laporte-Uribe, 2016; Laporte-Uribe, 2019; Laporte-Uribe, 2023). Therefore, measuring different CO2 species in rumen fluid is also recommended to address this inconsistency.

Perspectives for dealing with individual variability in rumen pH

Considering the high individual variation in susceptibility to the occurrence of low rumen pH, some consequences are expected for the proficiency of research and practical production. Therefore, some perspectives can be imagined for addressing this concept to improve the current conditions of research and practical production.

Research perspectives

Gaining knowledge concerning the mechanism, roles, and biomarkers (predictors) for the prediction of low rumen pH. As previously mentioned, more research is needed to determine which mechanism(s) are responsible for such differences in the rumen pH of similar animals fed the same diet (Khiaosa-ard *et al.* 2018; Nasrollahi *et al.* 2019; Hartinger *et al.* 2024).

Additionally, all the known indicators must be evaluated quantitatively to explain the proportional role of each indicator, which are the key indicators, and if the indicators are cause or effect (Pinheiro and Bates, 2000; Korb and Nicholson, 2010; Drury *et al.* 2017).

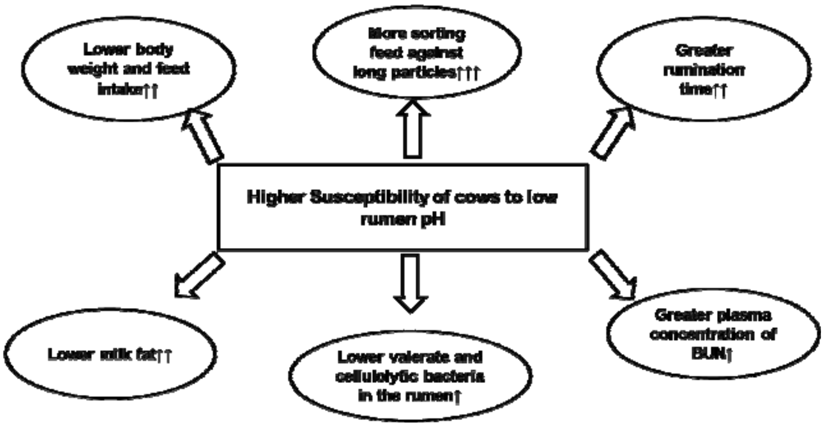

Figure 1 Characteristics of cows with increased susceptibility to low rumen pH. The numbers of ↑ indicate the strength of each effect

Table 1 Some characteristics of low rumen pH due to animal variability or dietary induction1

Category	Low-risk animals *vs.* high-risk animals				Control diet *vs.* induced diet			
Study	Gao and Oba (2014)	Gao and Oba (2016)	Nasrollahi *et al.* (2017)[2]	Khiaosa-ard *et al.* (2018)	Khafipour *et al.* (2009)[3, 4]	Li *et al.* (2012)[4]	Zhao *et al.* (2018)[3, 4]	Steele *et al.* (2012)[4]
Rumen pH char.								
Mean	0.45**	0.35**	0.61**	0.31**	0.20*	0.31*	0.82[a]	0.36**
Min.	0.61**	0.42**	0.76**	0.41**				0.71**
Duration pH<5.8	-546**	-456**	-842**	-672**	-161*	-300**	-333[a]	-619**
Area PH<5.8	-139**	-99**	338*	-	-87*	-79**		
DMI	1.8	1.1	1.9**	1.8	2.5**	-1.3		-1.3**
MF%	0.5	0.43	0.32*	1.09*	0.37**			0.56**
Acute phase protein in blood								
LPSBP				0.7	-34.9*	-0.6	-40**	
SAA				5	-383*		-260**	
HP				18.5	-484**		-220**	
Rumen volatile fatty acid (VFA) (mol/100 mol)								
A	0.7	-1	1.3		7.9*	-7.3	-21.8**	-1.8
P	-2.9	-0.7	-1.1		-12.3**	-9.2.	-8*	-7.4**
B	2.3		0.7		-3.4*	-4	-6.2**	-2.7**

[1] All values are the mean difference (means of low-risk cows- means of high-risk cows or mean of control diet- mean of low rumen pH-induced diets).
[2] For Nasrollahi *et al.* (2017), rumen pH-related values come from 14 animals, whereas other values are from 78 animals.
[3] In these experiments, a pH of 5.6 was used instead of 5.8.
[4] In these experiments, the VFA was the molar proportion instead of the molar percentage.
[*] and [**] indicate significant effects at 0.05 ≥ P > 0.01 and 0.01 ≥ P, respectively.
[a] No statistical comparison.

When all of these elements are disclosed, it is necessary to understand the real effect of such variability in the rumen pH on the variation in animal health, well-being, and performance (Korb and Nicholson, 2010; Drury *et al.* 2017). As mentioned in the previous sections, despite great variation in rumen pH, the variation was not accompanied by enough response in terms of health (inflammation) or performance (milk/milk fat production). On the other hand, as animals with low rumen pH are those with lower body weights and lower feed intake but produce similar milk compared with animals with high rumen pH (Nasrollahi *et al.* 2017), it might be premised that low rumen pH has a direct or indirect link with efficiency. However, at the same time, animals with a low rumen pH have a lower back fat thickness (Nasrollahi *et al.* 2019), which is an indicator of low reproductive performance (Mösenfechtel *et al.* 2002; Pires *et al.* 2017) and longevity (Cellini *et al.* 2019). Therefore, individual variability in the occurrence of low rumen pH can affect animal performance in a manner similar to a double-edged sword, and how much it can affect performance, which is an interfering factor, and the real (quantitative) effect of each one need to be determined via quantitative analysis (Pinheiro and Bates, 2000; Korb and Nicholson, 2010; Drury *et al.* 2017). To address such a question and conduct such a comprehensive quantitative analysis, a large amount of data on individual animals is needed, which can be obtained through infrastructural collaboration and combining individual data (Baumont *et al.* 2019). Additionally, the possible role of CO_2 species (Laporte-Uribe, 2016; Laporte-Uribe, 2019; Laporte-Uribe, 2023) should also be checked to determine whether the pH is at the center of the events.

After developing our understanding of susceptibility to the occurrence of low rumen pH and its relationship with animal performance and health, it is essential to find a way to measure rumen pH at the herd level in a large number of animals (Nasrollahi *et al.* 2019). The data for each animal can help to manage the problem through genetic selection and nutritional grouping (Howard, 2002; Nasrollahi *et al.* 2019). Owing to its cost and labor-intensive nature, direct measurement of the rumen pH at this level in many animals may not be possible; therefore, the next step would be finding appropriate biomarkers with appropriate equations to predict the susceptibility of each animal (Gao and Oba, 2015; Nasrollahi *et al.* 2019; Hartinger *et al.* 2024). This task has started in several research infrastructures, and several biomarkers have been evaluated (Gao and Oba, 2015; Nasrollahi *et al.* 2019; Stefańska *et al.* 2020). As detailed previously, blood concentrations of BHBA, urea-N, and aspartate aminotransferase are some of these promising biomarkers (Gao and Oba, 2015; Nasrollahi *et al.* 2019; Stefańska *et al.* 2020), but they are still not validated universally. Therefore, identifying appropriate biomarkers and prediction methods with defined interfering factors to separate cows accurately according to their susceptibility to low rumen pH is needed.

Development of nutritional and feeding strategies

Another role of research infrastructures is to explore the best nutritional strategies for animals with different susceptibilities to the occurrence of low rumen pH. Indeed, after finding an appropriate way of separating low-risk and high-risk animals, the next step would be finding the best management approach for each group (Cabrera *et al.* 2012; Contreras-Govea *et al.* 2015). Importantly, these strategies may not be the same as the general strategies for controlling diet-mediated low rumen pH, as discussed in Table 1. The nature of low rumen pH due to the diet is different from that of low rumen pH due to individual variability; thus, different solutions might be expected. Additionally, the optimum pH of high-risk and low-risk animals may not be the same; therefore, they may expect different diets to have optimum production efficiency (Zebeli *et al.* 2008). Therefore, the feeding requirements of these two groups may need to be reevaluated since some reports have shown a link between susceptibility to the occurrence of low rumen pH and the body size of animals (Nasrollahi *et al.* 2017). After nutrient formulation, feeding management also needs to be reconsidered because these two groups of animals exhibit different feeding behaviors (Gao and Oba, 2014; Macmillan *et al.* 2017; Nasrollahi *et al.* 2017). This step covers not only the physical form of the diet but also the way it is delivered (Macmillan *et al.* 2017).

Dealing with possible confounding effects with the treatment effect in other research experiments

The last point to mention for the important aspect of individual viability on rumen pH is how it may influence the effect of other treatments in other research experiments. This concern is crucial because such a large variation in rumen pH can be even greater than the variation caused by diet (see Table 1), and there is a high potential for interfering with the effects of experimental treatments (Coon *et al.* 2019). Indeed, as the animal is the experimental unit, individual variation increases the standard deviation (and standard error of the experiment) and the probability of type I error (Charan and Kantharia, 2013; Bujang and Baharum, 2016; Kaps and Lamberson, 2017). This is the case for both past and future studies and needs to be considered when evaluating the results of past experiments and designing new studies. Researchers need to find a way to obtain a trustworthy treatment effect, and amendments to both the experimental design and the statistical approach can be applied to remove or limit confounding effects (Charan and Kantharia, 2013; Bujang and Baharum, 2016; Kaps and Lamberson, 2017). Latin squares and other crossover designs are relatively safe because they eliminate animal effects (Kaps and Lamberson, 2017). A randomized design requires many animals to balance the effects of animal variability via random selection and rising power to address the high standard deviation in the rumen pH (Charan and Kantharia, 2013; Bujang and Baharum, 2016). To increase certainty, covariate measurement may also be helpful, but it is not easy to measure the rumen pH at the start of the experiment, and it does not satisfy the need for enough animals (Kaps and Lamberson, 2017). The good news for experimental diets with high forage is that the variation between animals is considerably smaller when animals are fed high-forage diets (Wetzels *et al.* 2017).

In addition, a statistical approach is suggested in which the diet effect is adjusted by normalizing and filtering the effects of animal variability, sensor deviation, and noise, which derives an indicator called the relative pH indicator (Villot *et al.* 2018). The results suggested a greater sensitivity of the relative indicators to acidosis, which explained the period of high starch consumption compared with the period of low starch consumption (Villot *et al.* 2018). However, the calculation needs a primary expectation that the animal variation happens randomly and that there is no interaction between the animal variation and the nature of the diet; however, as animals, depending on their tolerance level, respond differently to a high-starch diet (Khiaosa-ard *et al.* 2018), the correction for individual variability and diet change may not be satisfactory. Additionally, as the comparison of high-starch and low-starch diets was considered the standard for sensitivity analysis, it might not be appropriate because these two diets did not significantly affect the rumen LPS concentration (Villot *et al.* 2018) and may not cause a decrease in the rumen pH (Plaizier *et al.* 2008).

The mathematical/statistical approach may also be helpful for removing the interference effect of individual variability on rumen pH in past studies when they are combined with meta-analyses (i.e., meta-analyses of several experiments in which the pH of a limited number of animals was measured continuously). The suggestive approach has previously been applied for improving the correlation coefficient between variables with intraindividual variability (Liu *et al.* 1978) and for isolating the cow-specific part of residual feed intake from random error (Fischer *et al.* 2018). Although the suggestion is rather theoretical, variations between animals can be measured statistically when the dataset is large enough and when the same animals are measured repeatedly (Fischer *et al.* 2018). Under these conditions, by removing the variation between animals from the standard error, the remainder would be within animal variability, and finally, the treatment effect would be tested with minimal error (Kaps and Lamberson, 2017).

Farm (stockholders)

Grouping strategies

The grouping strategy is a practical way to address individual variations, and currently, animals on commercial farms are regularly grouped on the basis of some known and important variations, such as the amount of milk production and days in milk (Cabrera *et al.* 2012; Contreras-Govea *et al.* 2015; Cardoso *et al.* 2020). In some large farms, up to 20 groups of lactating cows are made (personal observation); therefore, there is a potential to grow on the basis of rumen pH (Nasrollahi *et al.* 2019; Huot *et al.* 2023). However, as mentioned, the important concern is what should be done with each group (low-risk *vs.* high-risk animals), which needs to be addressed by research. To date, only one study has attempted to address this question and has shown that increasing the feeding frequency from 1 to 3 can improve the rumen pH (pH<5.8 for 375 *vs.* 274 min/d for 1- *vs.* 3-time feedings) and milk fat percentage of high-risk animals (Macmillan *et al.* 2017). These recommendations may be of interest to commercial farms that feed only once or twice per day (von Keyserlingk *et al.* 2012; Sova *et al.* 2013), but since three or even four times of feeding is a regular practice for others (Esmaeili *et al.* 2016), it might not drive a new recommendation to manage high-risk cows. Notably, Macmillan *et al.* (2017) pointed to the role of excessive appetite in exacerbating the condition of high-risk animals. The condition of over-appetite may also occur when animals are delayed in their regular feeding time or fluctuate in their regular amount of feeding (King *et al.* 2016; Schwartzkopf-Genswein *et al.* 2003). However, it must be mentioned that extremes in any direction are problematic, and from another study dealing with individual variability, it was reported that feed intake in the early morning had a positive relationship with rumen pH (Figure 2; (Nasrollahi *et al.* 2016)). It is possible that factors that motivate cows to eat a regular amount of feed in the early morning help them manage low rumen pH (Nasrollahi *et al.* 2014; Niu and Harvatine, 2018), but the appropriate amount was not quantified. Despite the lack of clear recommendations for the nutrition and feeding of high-risk animals, separating them from low-risk animals at least provides the opportunity to gain information about them at the farm level, and direct observations may lead to the invention of some key practices to manage them.

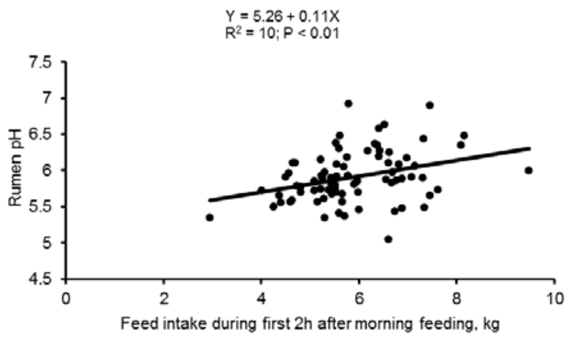

Figure 2 Regression relationship between rumen pH and feed intake during the first 2 h after morning feeding (Nasrollahi *et al.* 2016)

Genetic selection

Genetic selection is another management tool that allows homogenous individuals to facilitate and improve production. In this regard, variability in susceptibility to the occurrence of low rumen pH can be considered a chance to select resistant animals and reduce the problems related to the occurrence of low rumen pH (Penner and Beauchemin, 2010; Nasrollahi *et al.* 2019). However, as discussed previously, the concept of individual variability in the occurrence of low rumen pH is not a simple equation to be solved with one-direct selection. Compared with high-risk animals, low-risk animals have a large body size, with more feed intake but similar milk production (Nasrollahi *et al.* 2017), indicating that these animals spend more energy on maintenance than high-risk animals do (NRC, 2001) or mitigate more methane (Basarab *et al.* 2013). Therefore, the selection of low-risk animals may also be a selection for low-efficiency animals that are compromised. The addition of low rumen pH to the selection index and, on the basis of the overall economic value, the determination of animal selection may be beneficial.

CONCLUSION

This review summarized current findings on individual variability in susceptibility to low rumen pH in terms of causes and effects and provided possible strategies to manage it in research and commercial production systems. Substantial variability in rumen pH is detected among dairy cows with the same dietary and management conditions. Cows with a high incidence of low rumen pH are characterized by increased sorting of long particles in the diet; decreased body weight, feed intake and milk fat; and increased rumination time. The results concerning the rumen environment and blood metabolites indicate that some inconsistent effects on lower rumen apical absorption of VFAs and greater concentrations of some types of blood metabolism in these cows need more investigation. Moreover, these suggested mechanisms are not strong enough to cover the large variation in the rumen pH of animals fed the same diets, and more research is needed to evaluate the underlying mechanisms. Pioneering work has started to address individual variability at the commercial dairy farm level, including grouping strategies, animal selection, and increasing feeding frequency. However, this early evidence is not enough to make comprehensive recommendations, and these concepts need to be studied thoroughly. For research experiments, the individual variability in rumen pH can affect the results of other experiments. Therefore, it is suggested to measure the rumen pH at the beginning of each experiment to use it as a blocking factor or covariate, but the recommendation is also at the initial level and needs further evaluation.

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